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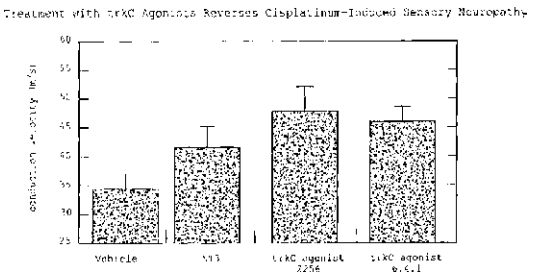
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(54)【発明の名称】アゴニスト抗－ＴＲＫ－Ｃモノクローナル抗体

(57)【要約】
本発明は、ｔｒｋＣの天然リガンドである、ＮＴ－３のある生物学的活性を模倣するアゴニスト抗－ｔｒｋＣモノクローナル抗体に関する。本発明は、さらに、、急性神経細胞系の損傷、及び末梢神経障害を含む慢性神経変性に関連する神経細胞損傷を含む細胞変性の予防及び／又は治療におけるそのような抗体の用途に関する。



【特許請求の範囲】
【請求項１】
（ａ）ｔｒｋＡ又はｔｒｋＢと顕著な交叉活性を示し；及び
（ｂ）ｔｒｋＣのドメイン５のエピトープを認識する、アゴニスト抗－ｔｒｋＣモノクローナル抗体。
【請求項２】
ｔｒｋＣのドメイン４のエピトープをさらに認識する請求項１の抗体。
【請求項３】
ヒト及びラットｔｒｋＣの双方に結合する請求項１の抗体。
【請求項４】
ヒト抗体である請求項１の抗体。
【請求項５】
マウス抗体である請求項１の抗体。
【請求項６】
ヒト化である請求項５の抗体。
【請求項７】
シスプラチン又はピリドキシン誘導神経障害の治療に効果的である、請求項１の抗体。
【請求項８】
糖尿病性神経障害の治療に効果的である、請求項１の抗体。
【請求項９】
患者へ投与した際に、痛覚過敏を起こさない請求項１の抗体。
【請求項１０】
ＮＴ－３と比較して増大した生体利用度を有する、請求項１の抗体。
【請求項１１】
ＮＴ－３より、より高い比活性を有する請求項１の抗体。
【請求項１２】
次のＣＤＲを含んでなる抗－ｔｒｋＣ抗体重鎖：配列番号：１，２，３，４及び５から成る群より選択されたＣＤＲ１；配列番号：６，７，８，９，１０及び１１から成る群より選択されたＣＤＲ２；及び配列番号：１２，１３，１４，１５，１６及び１７から成る群より選択されたＣＤＲ３。
【請求項１３】
次のＣＤＲを含んでなる抗－ｔｒｋＣ抗体軽鎖：配列番号：１８，１９，２０，２１，２２，２３及び２４から成る群より選択されたＣＤＲ１；配列番号：２５，２６，２７，２８，２９及び３０から成る群より選択されたＣＤＲ２；及び配列番号：３１，３２，３３，３４，３５及び３６から成る群より選択されたＣＤＲ３。
【請求項１４】
次のＣＤＲを含んでなるマウス抗－ｔｒｋＣ抗体重鎖：
（ａ）位置１のＸａａがＦ又はＹ；位置３のＸａａがＩ又はＭ；及び位置４のＸａａがＥ又はＨである、式ＸａａＷＸａａＸａａＷＶＫ（配列番号：３７）のＣＤＲ１；
（ｂ）位置３のＸａａがＬ又はＹ；位置５のＸａａがＧ又はＳ；位置６のＸａａがＳ又はＮ；位置７のＸａａがＤ又はＧ；位置８のＸａａがＮ又はＲ及び位置１６のＸａａがＧ又はＳである、式ＥＩＸａａＰＸａａＸａａＸａａＸａａＴＮＹＮＥＫＦＸａａ（配列番号：３８）のＣＤＲ２；及び
（ｃ）式ＫＮＲＮＹＹＧＮＹＶＶ（配列番号：１２）又はＫＹＹＹＧＮＳＹＲＳＷＹＦＤＶ（配列番号：１３）のＣＤＲ３。
【請求項１５】
次のＣＤＲを含んでなるヒト抗－ｔｒｋＣ抗体重鎖：
（ａ）位置１のＸａａがＳ又はＩ；位置２のＸａａがＧ又はＳ；位置３のＸａａがＧ，Ｔ又はＹ，及び位置７のＸａａがＳ又はＮである、式ＸａａＸａａＸａａＹＹＷＸａａ（配列番号：３９）のＣＤＲ１；

（ｂ）位置１のＸａａがＹ又はＲ；位置３のＸａａがＹ又はＦ；位置４のＸａａがＹ又はＴ；位置８のＸａａがＳ又はＲ；及び位置１０のＸａａがＮ又はＹである、式ＸａａＩＸａａＸａａＳＧＳＸａａＴＸａａＮＰＳＬＫＳ（配列番号：４０）のＣＤＲ２；及び（ｃ）位置８のＸａａがＡ又はＴ及び位置１６のＸａａがＤ又はＡである、ＤＲＤＹＤＳＴＧＤＹＹＳＹＹＧＭＤＶ（配列番号：１４）；ＤＧＧＹＳＮＰＦＤ（配列番号：１５）；ＥＲＩＡＡＡＧＸａａＤＹＹＹＮＧＬＸａａＶ（配列番号：４１）から成る群より選択された式のＣＤＲ３。

【請求項１６】

軽鎖と結合している請求項１４のマウス抗－ｔｒｋＣ重鎖のＣＤＲを含む重鎖を含んでなる抗－ｔｒｋＣアゴニストモノクローナル抗体。

【請求項１７】

ヒトフレームワーク残基を含んでなる請求項１６の抗体。

【請求項１８】

ｔｒｋＡ又はｔｒｋＢと顕著な交叉活性を示さない請求項１７の抗体。

【請求項１９】

２つのジスルフィド結合した抗体重鎖－軽鎖対からなるホモ４量体構造を有する請求項１７の抗体。

【請求項２０】

Ｆｖ，Ｆａｂ及び$F(ab')_2$断片から成る群より選択された抗体断片である、請求項１７の抗体。

【請求項２１】

ヒト抗体軽鎖と結合した請求項１５のヒト抗－ｔｒｋＣ抗体重鎖を含んでなる抗－ｔｒｋＣアゴニストモノクローナル抗体。

【請求項２２】

ｔｒｋＡ又はｔｒｋＢと顕著な交叉活性を示さない請求項２１の抗体。

【請求項２３】

２つのジスルフィド結合した抗体重鎖－軽鎖対からなるホモ４量体構造を有する請求項２２の抗体。

【請求項２４】

Ｆｖ，Ｆａｂ及び$F(ab')_2$断片から成る群より選択された抗体断片である、請求項２２の抗体。

【請求項２５】

ＩｇＧである請求項２２の抗体。

【請求項２６】

ＩｇＧ－２又はＩｇＧ－４である請求項２５の抗体。

【請求項２７】

抗体２２４８，２２５０，２２５３及び２２５６から成る群より選択されたマウス抗－ｔｒｋＣアゴニスト抗体。

【請求項２８】

抗体６．１．２，６．４．１，２３４５，２３４９及び２３４４から成る群より選択されたヒト抗－ｔｒｋＣアゴニスト抗体。

【請求項２９】

抗体６．１．２，６．４．１，２３４５及び２３４９から成る群より選択された請求項２８の抗体。

【請求項３０】

請求項２７のマウス抗－ｔｒｋＣアゴニスト抗体をコードする単離された核酸分子。

【請求項３１】

請求項２８のヒト抗－ｔｒｋＣアゴニスト抗体をコードする単離された核酸分子。

【請求項３２】

ＰＴＡ－２１３６，ＰＴＡ－２１３７及びＰＴＡ－２１３８から成る群より選択された寄

託番号の下へ、２０００年６月２１日にＡＴＣＣに寄託された核酸分子。
【請求項３３】
ＰＴＡ－２１３３、ＰＴＡ－２１３４、ＰＴＡ－２１３５、ＰＴＡ－２１３６、ＰＴＡ－２１３９、ＰＴＡ－２１４０、ＰＴＡ－２１４１、ＰＴＡ－２１４２及びＰＴＡ－２１４３から成る群より選択された寄託番号の下で、２０００年６月２１日にＡＴＣＣに寄託された核酸分子。
【請求項３４】
請求項３０－３３の任意の核酸分子を含んでなるベクター。
【請求項３５】
請求項３０－３３の任意の核酸分子で形質転換された宿主細胞株。
【請求項３６】
請求項３０－３３の任意の核酸分子で形質転換されたハイブリドーマ細胞株。
【請求項３７】
請求項３６のハイブリドーマ細胞株によって生産された抗体。
【請求項３８】
抗－ｔｒｋＣアゴニストモノクローナルの重鎖をコードする単離された核酸分子であって、配列番号：５８，配列番号：６０，配列番号：６２，配列番号：６４，配列番号：６６，配列番号：６８及び配列番号：７０から成る群より選択された前記核酸分子。
【請求項３９】
抗－ｔｒｋＣアゴニストモノクローナルの軽鎖をコードする単離された核酸分子であって、配列番号：５９，配列番号：６１，配列番号：６３，配列番号：６５，配列番号：６７，配列番号：６９及び配列番号：７１から成る群より選択された前記核酸分子。
【請求項４０】
請求項３８又は請求項３９の核酸分子を含んでなるベクター。
【請求項４１】
請求項３８又は請求項３９の核酸分子で形質転換された宿主細胞。
【請求項４２】
請求項３８の核酸分子及び請求項３９の核酸分子の双方で形質転換された宿主細胞。
【請求項４３】
請求項３８又は請求項３９の核酸分子で形質転換されたハイブリドーマ細胞株。
【請求項４４】
請求項３８の核酸分子及び請求項３９の核酸分子で形質転換されたハイブリドーマ細胞株。
【請求項４５】
請求項４３のハイブリドーマ細胞株によって生産された抗体。
【請求項４６】
請求項４４のハイブリドーマ細胞株によって生産された抗体。
【請求項４７】
請求項３８又は３９の核酸分子によってコードされているポリペプチド。
【請求項４８】
請求項３８の核酸分子及び請求項３９の核酸分子によってコードされているポリペプチド鎖を含んでなるポリペプチド。
【請求項４９】
薬学的に許容可能な担体との混合物である、請求項１，１６，２１の任意のアゴニスト抗－ｔｒｋＣモノクローナル抗体の有効量を含んでなる薬学的組成物。
【請求項５０】
請求項１の抗体の有効量を哺乳動物へ投与することを含んでなる、神経障害又は神経変性疾患の治療、又は損傷した神経細胞を修復するために方法。
【請求項５１】
末梢神経障害、糖尿病性神経障害、及び多繊維状感覚神経障害から成る群から選択された

前記神経障害である、請求項５０の方法。
【請求項５２】
前記神経変性疾患が筋萎縮性側索硬化症（ＡＬＳ）である、請求項５０の方法。
【請求項５３】
前記神経細胞が感覚ニューロン又は運動ニューロンである、請求項５０の方法。
【請求項５４】
前記感覚ニューロンが背根神経節からである、請求項５３の方法。
【請求項５５】
前記運動ニューロンが脊髄である、請求項５３の方法。
【請求項５６】
前記投与が静脈注射又は皮下注射である、請求項５０の方法。
【請求項５７】
前記投与が局所的である、請求項５０の方法。
【請求項５８】
請求項１，請求項１６，又は請求項２１の抗体の有効量と前記ニューロンを接触させることを含んでなる、末梢ニューロンの増殖、維持又は再生を向上させる方法。
【請求項５９】
細胞変性に関する疾病又は症状のそのような被検体の細胞へ請求項１の抗－ｔｒｋＣ抗体をコードする核酸を導入することによって、そのような哺乳動物被検体を治療する方法。
【請求項６０】
神経障害、神経変性疾患、及び神経細胞損傷から成る群から選択された疾患又は症状である、請求項５９の方法。
【請求項６１】
前記細胞が神経細胞である、請求項５９の方法。
【請求項６２】
前記核酸をエキソビボで前記細胞へ導入する、請求項５９の方法。
【請求項６３】
前記核酸をインビボで前記細胞へ導入する、請求項５９の方法。
【請求項６４】
血管形成を誘導するのに有効量の請求項１の抗－ｔｒｋＣ抗体を送達することを含んでなる、アゴニスト抗－ｔｒｋＣ抗体を生成する方法。
【請求項６５】
ｔｒｋＣの天然ＮＴ－３リガンドの結合部位と重なり合う部位と特異的に結合する抗体を調製することを含んでなる、アゴニスト抗－ｔｒｋＣ抗体を生成する方法。
【請求項６６】
前記ｔｒｋＣの天然ＮＴ－３リガンドと基本的に同じ部位で前記抗体がｔｒｋＣと結合する、請求項６５の方法。
【請求項６７】
前記抗体が、ｔｒｋＣのドメイン５の中のエピトープと結合する、請求項６５の方法。
【請求項６８】
前記ｔｒｋＣが天然ヒトｔｒｋＣポリペプチドである、請求項６７の方法。
【請求項６９】
前記エピトープが、前記天然ヒトｔｒｋＣのアミノ酸残基Ｌ２８４，Ｅ２８７，及びＮ３３５を含む、請求項６８の方法。
【発明の詳細な説明】
【０００１】
（発明の背景）
（発明の分野）
この発明は、アゴニスト抗－ｔｒｋＣ モノクローナル抗体に関する。更に、急性神経細胞系の傷害に関連する神経細胞損傷、及び末梢神経障害を含む慢性神経変性疾患を含む細

胞変性症の予防及び／又は治療でのアゴニスト抗体の用途に関する。

【０００２】

（関連するＡｒｔの記述）

ニューロトロフィンは、小さな、塩基性タンパク質のファミリーで、神経系の発達と維持に重要な役割を果たす。最初に同定され、恐らくは最も理解されているこのファミリーのメンバーは、末梢神経系の知覚性及び交換神経の発達に顕著な影響を与える神経成長因子（ＮＧＦ）である（Ｌｅｖｉ－Ｍｏｎｔａｌｃｉｎｉ，Ｒ．及びＡｎｇｅｌｅｔｔｉ，Ｐ．Ｕ．，Ｐｈｙｓｉｏｌ．Ｒｅｖ．４８，５３４－５６９［１９６８］；Ｔｈｏｅｎｅｎ，Ｈ．ら．，Ｒｅｖ．Ｐｈｙｓｉｏｌ．Ｂｉｏｃｈｅｍ．Ｐｈａｒｍａｃｏｌ．１０９，１４５－１７８［１９８７］）。ＮＧＦは長きにわたって知られているが、マウス顎下腺の相同体、成熟、－又は２．５Ｓ ＮＧＦの活性型としばしば言われているものを含めて、経時的に関連するが、類似する機能を有する異なるポリペプチドが同定された。

最初に整えられたのは、脳由来神経栄養因子（ＢＤＮＦ）と呼ばれる因子で、Ｌｅｉｂｒｏｃｋ，Ｊ．ら．（Ｎａｔｕｒｅ ３４１，１４９－１５２［１９８９］）によってクローン化されて配列が決定された。この因子は、最初はブタ脳から精製され（Ｂａｒｄｅ，Ｙ．Ａ．ら．，ＥＭＢＯ Ｊ．１，５４９－５５３［１９８２］）たが、そのＮＧＦとの相同性が明かになったのは、そのｃＤＮＡがクローン化されて配列が決定されてからであった。ＮＧＦとＢＮＤＦとの間の全体的なアミノ酸配列同一性は約５０％である。この発見の見地から、Ｌｅｉｂｒｏｃｋらは、ＢＤＮＦとＮＧＦが、共通の構造と機能特性を有するニューロトロフィンのファミリーの唯一のメンバーであると考える理由がないことを推測した。

【０００３】

実際に、ＮＧＦ及びＢＤＮＦに極めて関連する更なるニューロトロフィンが見出されている。幾つかのグループが、現在はニューロトロフィン－３（ＮＴ－３）と呼ばれている、元々神経細胞因子（ＮＦ）と呼ばれるニューロトロフィンを同定した（Ｅｒｎｆｏｒｔｓら．，Ｐｒｏｃ．Ｎａｔｌ．Ａｃａｄ．Ｓｃｉ．ＵＳＡ ８７，５４５４－５４５８（１９９０）；Ｈｏｈｎら．，Ｎａｔｕｒｅ ３４４，３３９［１９９０］；Ｍａｉｓｏｎｐｉｅｒｒｅら．，Ｓｃｉｅｎｃｅ ２４７，１４４６［１９９０］；Ｒｏｓｅｎｔｈａｌら．，Ｎｅｕｒｏｎ ４，７６７［１９９０］；Ｊｏｎｅｓ及びＲｅｉｃｈａｒｄｔ，Ｐｒｏｃ．Ｎａｔｌ．Ａｃａｄ．Ｓｃｉ．ＵＳＡ ８７，８０６０－８０６４（１９９０）；Ｋａｉｓｈｏら．，ＦＥＢＳ Ｌｅｔｔ．２６６，１８７［１９９０］）。ＮＴ－３は、そのアミノ酸の約５０％をＮＧＦ及びＢＤＮＦ（ＮＴ－２）の双方と共有する。ニューロトロフィン－４及び－５（ＮＴ－４及びＮＴ－５）が、ファミリーに加えられた（１９９４年１１月１５日に発行の米国特許第５，３６４，７６９号；Ｈａｌｌｂｏｏｋ，Ｆ．ら．，Ｎｅｕｒｏｎ ６，８４５－８５８［１９９１］；Ｂｅｒｋｍｅｉｅｒ，Ｌ．Ｒ．ら．，Ｎｅｕｒｏｎ ７，８５７－８６６［１９９１］；Ｉｐら．，Ｐｒｏｃ．Ｎａｔｌ．Ａｃａｄ．Ｓｃｉ ＵＳＡ ８９，３０６０－３０６４［１９９２］）。その後にアフリカツメガエルのＮＴ－４の相同体と見られた、Ｂｅｒｋｍｅｉｅｒら，上掲によって最初に記載された哺乳動物の分子は、通常はＮＴ－４／５と呼ばれている。更には、ＮＴ－６と呼ばれている酸性相同性タンパク質と記載されているものが哺乳動物にある（Ｂｅｒｋｅｍｅｉｒ，ら．，Ｓｏｍａｔ．Ｃｅｌｌ Ｍｏｌ．Ｇｅｎｅｔ．１８（３）：２３３－２４５［１９９２］）。更に最近では、魚、クシフォフォルスのその他の相同タンパク質もＮＴ－６と表示されている（Ｇｏｔｚら．，Ｎａｔｕｒｅ ３７２：２６６－２６９［１９９４］）。ＮＴ－７として記載されている２つのタンパク質が文献にあり、その一つは鯉、Ｃｙｐｒｉｎｕｓから（Ｌａｉ，ら．，Ｍｏｌ．Ｃｅｌｌ．Ｎｅｕｒｏｓｃｉ．１１（１－２）：６４－７６（１９９８））、そしてもう一つはゼブラフィッシュ、ダニオからクローン化された（Ｎｉｌｓｓｏｎら．，ＦＥＢＳ Ｌｅｔｔｅｒｓ ４２４（３）：２８５－９０［１９９８］）。この前出に記載の三種の魚のニューロトロフィンは、魚以外では記載がなく、任意の哺乳動

物のニューロトロフィンとそれらとの関係は不明である。ゼブラフィッシュのニューロトロフィン－７（ｚＮＴ－７）のアミノ酸配列は、その他の任意のニューロトロフィンのそれよりも、魚の神経成長因子（ＮＧＦ）及びニューロトロフィン－６（ＮＴ－６）のそれにより近接に関連している。しかしながら、ｚＮＴ－７は、魚のＮＧＦ及びＮＴ－６に等しく関連していて（それぞれ、６５％及び６３％アミノ酸配列同一性）、それが異なるニューロトロフィン配列を表していることを示唆している。ｚＮＴ－７は、成熟タンパク質の中間のベーターターン領域で１５アミノ酸残基を含有している。組み換えｚＮＴ－７は、ラットＮＧＦよりも効率が低いにも関わらず、ヒトｐ７５ニューロトロフィンレセプターと結合し、ラットｔｒｋＡレセプターチロシンキナーゼのチロシンリン酸化を誘導することが可能であった。ｚＮＴ－７は、ラットｔｒｋＢ又はｔｒｋＣと相互作用せず、ＮＧＦのような類似のレセプター特異性を示した。我々は、ニューロトロフィンの進化系統樹におけるＮＧＦサブファミリーの多様化が、ｚＮＴ－７及びＮＴ－６のような幾つかの更なるメンバーの出現した硬骨魚類の進化の間に起こり、構造及び機能的にＮＧＦと関連していることを提案した。

【０００４】

他のポリペプチド成長因子に類似するニューロトロフィンは、細胞表面レセプターとの相互作用を介してそれらの標的細胞へ作用する。我々の現在の知識によると、二種類の膜貫通糖タンパク質がニューロトロフィンのレセプターとして機能を果たす。平衡結合研究によって、ニューロトロフィン－応答ニューロンが、ＮＧＦ、ＢＤＮＦ、及びＮＴ－３と２ｘ１０$^{-8}$ＭのＫ$_{D}$で結合する、ｐ７５^{NTR}とも呼ばれる共通の低分子量（６５－８０ｋＤａ）、低親和性レセプター（ＬＮＧＦＲ）、及びレセプターチロシンキナーゼのｔｒｋファミリーのメンバーで、高分子量（１３０－１５０ｋＤａ）、高親和性（Ｋ$_{D}$が１０$^{-11}$Ｍ）を有することが示された。

ｔｒｋレセプターファミリーの最初のメンバーであるｔｒｋＡは、トロポミオシンの触媒ドメインへのトロポミオシン配列のトランスロケーションによって生じた癌遺伝子の形質転換の結果であると最初に同定された（Ｍａｒｔｉｎ－Ｚａｎｃａら．，Ｍｏｌ．Ｃｅｌｌ．Ｂｉｏｌ．９（１）：２４－３３（１９８９））。後の研究によって、ｔｒｋＡがＮＧＦのシグナル伝達レセプターとして同定された。続いて、二つの他の関連レセプターである、マウス及びラットのｔｒｋＢ（Ｋｌｅｉｎら．，ＥＭＢＯ Ｊ．８，３７０１－３７０９（１９８９））；Ｍｉｄｄｌｅｍａｓら．，Ｍｏｌ．Ｃｅｌｌ．Ｂｉｏｌ．１１，１４３－１５３［１９９１］；１９９３年1月１３日発行のＥＰ ５２２，５３０）及びブタ、マウス及びラットｔｒｋＣ（Ｌａｍｂａｌｌｅら．，Ｃｅｌｌ ６６，９６７－９７９［１９９１］；１９９３年1月１３日発行のＥＰ ５２２，５３０）が、ｔｒｋレセプターファミリーのメンバーとして同定された。ｔｒｋレセプターの構造は極めて類似しているが、ｔｒｋＡの二つの既知の型、ｔｒｋＢの三つの既知の型（二つは機能的チロシンキナーゼドメインを有しない）、及びｔｒｋＣの少なくとも四つの型（幾つかは機能的チロシンキナーゼドメインを有せず、そして二つはチロシンキナーゼドメインに小さな挿入を有する）を生ずることによって、選択的スプライシングはファミリーの複雑さを増す。

【０００５】

ｐ７５及びｔｒｋレセプターの役割は議論のあるところである。一般的には、ｔｒｋレセプターチロシンキナーゼが特定のニューロトロフィンへ結合特異性を付与することにおいて重要な役割を担うということが受け入れられているが、しかしながら、ＮＴ－３のみ（しかし、他のニューロトロフィンではない）と結合すると報告されていたｔｒｋＣ発現細胞とは対照的に、ｔｒｋＡを発現する細胞株はＮＧＦだけでなく、ＮＴ－３及びＮＴ－４／５（しかしＢＤＮＦではない）にも結合し、ｔｒｋＢを発現する細胞は、ＢＤＮＦ、ＮＴ－３、ＮＴ－４及びＮＴ－４／５（しかしＮＧＦではない）と結合する。更には、選択的スプライシングによって生じるｔｒｋレセプターの種々の型が、異なる細胞内シグナル経路を活性化し、それ故に、恐らくはインビボで異なる生理学的機能を媒介するということがモデル系で示されている。ｐ７５が存在せずに、特定のｔｒｋレセプターを発現する

細胞がニューロトロフィンと低又は高親和性で結合するかどうかは不明である（Ｍｅａｋｉｎ及びＳｈｏｏｔｅｒ，Ｔｒｅｎｄｓ Ｎｅｕｒｏｓｃｉ．１５，３２３－３３１［１９９２］）。

【０００６】

種々の細胞株を用いた研究の公開された結果はわかりにくく、ｐ７５は、ニューロトロフィンの応答性にとっては必修又は不要であるかのいずれかであると示唆している。ｐ７５のみを発現する細胞株は、平衡時での同じような低親和性でＮＧＦ、ＢＤＮＦ、ＮＴ－３及びＮＴ－４と結合するが、結合速度常数は明らかに異なる。結果として、ｐ７５結合はすべてのニューロトロフィンの共通特性であるが、ｐ７５レセプターはまた、リガンド識別において役割を担うが示唆されていた（Ｒｏｄｒｉｇｕｅｚ－Ｔｅｂａｒら．，ＥＭＢＯ Ｊ．１１，９１７－９２２［１９９２］）。ｔｒｋレセプターが伝統的に生物学的に重要なニューロトロフィンレセプターとして考えられている一方で、ｔｒｋＡ発現を欠くメラノーマ細胞では、ＮＧＦは，まだ、恐らくはｐ７５を介して生物学的行動の多大な変化を誘導することができる（Ｈｅｒｍａｎｎら．，Ｍｏｌ．Ｂｉｏｌ．Ｃｅｌｌ ４，１２０５－１２１６［１９９３］）。Ｄａｖｉｅｓら．（Ｎｅｕｒｏｎ １１，５６５－５７４［１９９３］）は、ｐ７５遺伝子に無発現変異を有するトランスジェニックマウスのモデルにおける、ニューロトロフィンへの胚ニューロンの生存応答をの媒介におけるｐ７５の役割を調べる研究の結果を報告している。彼らは、ｐ７５が、ＮＧＦへのＮＧＦ－依存性皮膚反応感覚ニューロンの感度を増すことを見出した。ｐ７５が、ニューロトロフィンの少なくとも幾つかの生物学的効果を媒介することができることを、現在では多くの研究が示している。この分野には、いまだに若干議論があるが、ｐ７５シグナリングは、細胞死をコントロールすること及び神経突起成長に関与している（Ｂａｒｋｅｒ，ＰＡ，Ｃｅｌｌ Ｄｅａｔｈ Ｄｉｆｆ．５：３４６－３５６［１９９８］；Ｂｒｅｄｅｓｅｎら．，Ｃｅｌｌ Ｄｅａｔｈ Ｄｉｆｆ．５：３５７－３６４［１９９８］；Ｃａｓａｃｃｉａ－Ｂｏｎｎｅｆｉｌ，ら．，Ｃｅｌｌ Ｄｅａｔｈ Ｄｉｆｆ．５：３５７－３６４［１９９８］；Ｒａｏｕｌら．，Ｃｕｒｒ．Ｏｐ．Ｎｅｕｒｏｂｉｏｌ．１０：１１１－１１７［２０００］；Ｄａｖｉｅｓ，ＡＭ，Ｃｕｒｒ．Ｂｉｏｌ．１０：Ｒ１９８－Ｒ２００［２０００］）。重要なことには、ｐ７５の刺激が、ｔｒｋＣを刺激することの効果を改良するとが示されている（Ｈａｐｎｅｒ，ら．，Ｄｅｖｅｌｏｐｍ．Ｂｉｏｌ．２０１：９０－１００［１９９８］）。

【０００７】

完全長天然ｔｒｋＡ、ｔｒｋＢ及びｔｒｋＣレセプターの細胞外ドメインは、５つの機能的ドメインを有し、相同性又はそうでなければ種々の他のタンパク質で同定された類似の構造に関連して特徴付けられている。ドメインは、成熟ｔｒｋレセプターのアミノ酸配列のＮ末端から、１）ヒトｔｒｋＡのアミノ酸位置１から約アミノ酸位置３２、ヒトｔｒｋＢのアミノ酸位置１から約アミノ酸位置３６、及びヒトｔｒｋＣのアミノ酸位置１から約アミノ酸位置４８へ延びる最初のシステインリッチドメイン；２）ｔｒｋＡの約アミノ酸３３から約アミノ酸１０４、ｔｒｋＢの約アミノ酸３７から約アミノ酸１０８、及びｔｒｋＣの約アミノ酸４９から約アミノ酸１２０へ伸びるロイシンリッチドメイン；３）ｔｒｋＡの約アミノ酸１０５から約アミノ酸１５７；ｔｒｋＢの約アミノ酸１０９から約アミノ酸１６４；及びｔｒｋＣの約アミノ酸１２１から約アミノ酸１７７の二番目のシステインリッチドメイン；４）ｔｒｋＡの約アミノ酸１７６から約アミノ酸２３４；ｔｒｋＢの約アミノ酸１８３から約アミノ酸２３９；及びｔｒｋＣの約アミノ酸１９６から約アミノ酸２５７へ伸びる最初の免疫グロブリン様ドメイン；５）ｔｒｋＡの約アミノ酸２６４から約アミノ酸３３０、ｔｒｋＢの約アミノ酸２７０から約アミノ酸３３４；ｔｒｋＣの約アミノ酸２８８から約アミノ酸３５１へ延びる二番目の免疫グロブリン様ドメインとして開始して示される。

【０００８】

ニューロトロフィンは、異なるが重なり合っている末梢及び中枢ニューロンの組に作用を示す。これらの効果は、発達しているニューロン（感覚及び交感神経ニューロンのＮＧＦ

）の生存を確かにするのに重要な役割を担うことから、ニューロンの形態学（プルキンエ細胞のＮＴ－３）への比較的に僅かの効果までに及ぶ。これらの活性が、ニューロトロフィンをある神経変性疾患の治療法に用いることの関心へつながった。ＮＴ－３には、また、末梢血白血球の増殖を促進することが見出され、結果として、ＮＴ－３が、好中球減少、感染症疾患及び腫瘍の治療に用いることができることが示唆された（２０００年６月１８日に発行の米国特許第６，０１５，５５２号）。

心臓血管の発達の制御、損傷に対する血管性応答の調節におけるニューロトロフィンの役割も調べられてきた（Ｄｏｎｏｖａｎら．，Ｎａｔｕｒｅ Ｇｅｎｅｔｉｃｓ １４：２１０－２１３［１９９６］；Ｄｏｎｏｖａｎら．，Ａ．Ｊ．Ｐａｔｈ．１４７：３０９－３２４［１９９５］；Ｋｒａｅｍｅｒら．，Ａｒｔｅｒｉｏｌ．Ｔｈｒｏｍｂ，ａｎｄ Ｖａｓｃ．Ｂｉｏｌ．１９：１０４１－１０５０［１９９９］）。ニューロトロフィンは、血管形成及び血管の健全性を制御するための潜在的な療法として記載されている（ＰＣＴ発行国際公開００／２４４１５、２０００年５月４日発行）。

【０００９】

細胞性変性の治療におけるニューロトロフィンの裏付けにも関わらず、例えば神経変性疾患及び急性ニューロン損傷、及び潜在的な血管形成のために生じる疾患のために、ニューロトロフィンには幾つかの欠点がある。一つの重要な欠点は、特異性を欠くことである。殆どのニューロトロフィンは、一つより多いレセプターと交差する。例えば、ｔｒｋＣレセプターチロシンキナーゼの好ましいリガンドであるＮＴ－３は、ｔｒｋＡ及びｔｒｋＢとも結合して活性化する（Ｂａｒｂａｃｉｄ，Ｊ．Ｎｅｕｒｏｂｉｏｌ．２５：１３８６－１４０３［１９９４］；Ｂａｒｂａｒｃｉｄ，Ａｎｎ．Ｎｅｗ Ｙｏｒｋ Ａｃａｄ．Ｓｃｉ．７６６：４４２－４５８［１９９５］；Ｒｙｄｅｎ及びＩｂａｎｅｚ，Ｊ．Ｂｉｏｌ．Ｃｈｅｍ．２７１：５６２３－５６２７［１９９６］；Ｂｅｌｌｉｖｅａｕら．，Ｊ．Ｃｅｌｌ．Ｂｉｏｌ．１３６：３７５－３８８［１９９７］；Ｆａｒｉｎａｓら．，Ｎｅｕｒｏｎ ２１：３２５－３３４［１９９８］）。結果として、ニューロンの特定の集団を標的とする療法を考案することは困難である。ニューロトロフィン療法のその他の限界としては、ＮＴ－３が痛覚過敏を導くということが知られていることを含むニューロトロフィンのことである（Ｃｈａｕｄｈｒｙ，ら．，Ｍｕｓｃｌｅ ａｎｄ Ｎｅｒｖｅ ２３：１８９－１９２［２０００］）。更には、ＮＴ－３のような幾つかのニューロトロフィンは、それらのヒト臨床応用について深刻な疑問を生じさせる、げっ歯類での貧弱な薬動力学及び生体利用度特性を有する（Ｈａａｓｅら．，Ｊ．Ｎｅｕｒｏｌ．Ｓｃｉ．１６０：Ｓ９７－Ｓ１０５［１９９８］，投与量は、Ｈｅｌｇｒｅｎら．，Ｊ．Ｎｅｕｒｏｓｃｉ．１７（１）：３７２－８２［１９９７］で使用され、データが下記にあり）。

従って、既知のニューロトロフィンの欠点を欠く、神経変性疾患及び急性神経細胞損傷の治療のための新しい治療薬の開発に関する多大な必要性が存在する。

【００１０】

（発明の要約）

現在の発明は、ＮＴ－３の生物学的活性、ｔｒｋＣレセプターの天然リガンドを模倣するが、幾つかのＮＴ－３の既知の損害の無い、ｔｒｋＣレセプターの細胞外ドメインにあるエピトープに対して向けられた、アゴニスト抗－ｔｒｋＣモノクローナル抗体の開発と特徴付けに基づく。本発明は、また、実験動物モデルでの神経障害の治療におけるこれらアゴニスト抗体の有用性を示す。抗－ｔｒｋＣアゴニスト抗体は、細胞変性症、例えば神経細胞損傷、特に末梢神経障害又は幾つか列挙するとトラウマ、毒剤、手術、等の外的因子に起因するような神経変性疾患に関連する神経細胞損傷の予防又は治療法でのＮＴ－３に多大な有利な点を与える。

【００１１】

一側面では、本発明は、（ａ）ｔｒｋＡ又はｔｒｋＢと顕著な交差を示さない；及び（ｂ）ｔｒｋＣのドメイン５のエピトープを認識する、アゴニスト抗－ｔｒｋＣモノクローナル抗体に関する。

【００１２】
本発明のあるアゴニスト抗体は、ｔｒｋＣのドメイン４のエピトープをさらに認識する。好ましい実施態様では、この抗体はヒト及びげっ歯類（例えば、ラット又はマウス）ｔｒｋＣの双方に結合し、マウス、キメラ（ヒト化を含む）又はヒト抗体も結合し得るであろう。この抗体は、天然ｔｒｋＣリガンド、ＮＴ－３の少なくとも一つの活性を模倣し、従って、細胞変性を含む種々の疾患の予防及び／又は治療にとって効果的であり、その細胞変性には、例えば、シスプラチン－又はピリドキシン誘導神経障害、糖尿病性神経障害のような神経障害、そして（細胞変性には、骨髄細胞変性が含まれる）血液細胞不足の疾患、例えば好酸球減少症、及び／又は好塩基球減少症、リンパ球減少症、単球減少症、及び好中球減少症が含まれる。

その他の側面では、本発明は、次のＣＤＲを含む抗－ｔｒｋＣ抗体重鎖に関する：配列番号：１，２，３，４及び５からなる群から選択されるＣＤＲ１；配列番号：６，７，８，９，１０及び１１からなる群から選択されるＣＤＲ２；及び配列番号：１２，１３，１４，１５，１６及び１７からなる群から 選択されるＣＤＲ３。

【００１３】
更にその他の側面では、本発明は、次のＣＤＲを含む抗－ｔｒｋＣ抗体軽鎖に関する：配列番号：１８，１９，２０，２１，２２，２３及び２４から成る群より選択されたＣＤＲ１；配列番号：２５，２６，２７，２８，２９及び３０から成る群より選択されたＣＤＲ２；配列番号：３１，３２，３３，３４，３５及び３６から成る群より選択されたＣＤＲ３。

更なる側面では、本発明は、次のＣＤＲを含むマウス抗－ｔｒｋＣ抗体重鎖に関する：
（ａ）位置１のＸａａがＦ又はＹ；位置３のＸａａがＩ又はＭ；及び位置４のＸａａがＥ又はＨである、式ＸａａＷＸａａＸａａＷＶＫ（配列番号：３７）のＣＤＲ１；
（ｂ）位置３のＸａａがＬ又はＹ；位置５のＸａａがＧ又はＳ；位置６のＸａａがＳ又はＮ；位置７のＸａａがＤ又はＧ；位置８のＸａａがＮ又はＲ及び位置１６のＸａａがＧ又はＳである、式ＥＩＸａａＰＸａａＸａａＸａａＸａａＴＮＹＮＥＫＦＸａａ（配列番号：３８）のＣＤＲ２；及び
（ｃ）式ＫＮＲＮＹＹＧＮＹＶＶ（配列番号：１２）又はＫＹＹＹＧＮＳＹＲＳＷＹＦＤＶ（配列番号：１３）のＣＤＲ３。

【００１４】
より更なる側面では、本発明は、次のＣＤＲを含むヒト抗－ｔｒｋＣ抗体重鎖に関連する：
（ａ）位置１のＸａａがＳ又はＩ；位置２のＸａａがＧ又はＳ；位置３のＸａａがＧ，Ｔ又はＹ，及び位置７のＸａａがＳ又はＮである、式ＸａａＸａａＸａａＹＹＷＸａａ（配列番号：３９）のＣＤＲ１；
（ｂ）位置１のＸａａがＹ又はＲ；位置３のＸａａがＹ又はＦ；位置４のＸａａがＹ又はＴ；位置８のＸａａがＳ又はＲ；及び位置１０のＸａａがＮ又はＹである、式ＸａａＩＸａａＸａａＳＧＳＸａａＴＸａａＮＰＳＬＫＳ（配列番号：４０）のＣＤＲ２；及び
（ｃ）位置８のＸａａがＡ又はＴ及び位置１６のＸａａがＤ又はＡである、ＤＲＤＹＤＳＴＧＤＹＹＳＹＹＧＭＤＶ（配列番号：１４）；ＤＧＧＹＳＮＰＦＤ（配列番号：１５）；ＥＲＩＡＡＡＧＸａａＤＹＹＹＮＧＬＸａａＶ（配列番号：４１）から成る群より選択された式のＣＤＲ３。

【００１５】
その他の側面では、本発明は、軽鎖に関連した請求項１４のマウス抗－ｔｒｋＣ抗体重鎖のＣＤＲを含んでなる抗－ｔｒｋＣアゴニストモノクローナル抗体に関する。この抗体は、好ましくはヒト又はヒトフレームワーク残基を含み、そして好ましくは、ｔｒｋＡ又はｔｒｋＢとの顕著な交差を示さない。この出願を通して、抗体は広い意味で定義され、そして特に抗体断片、例えばＦｖ断片、Ｆａｂ断片、Ｆａｂ’又はＦ（ａｂ’）$_2$断片を含む。抗体のすべてのクラス及びアイソタイプが含まれるが、ＩｇＧ、特にＩｇＧ－２及びＩｇＧ－４が好まれる。

更にその他の側面では、本発明は、マウス又はヒト抗－ｔｒｋＣアゴニスト抗体重鎖又は軽鎖、又はその断片をコードする単離された核酸に関する。特定の実施態様では、この核酸は、ＰＴＡ－２１３３、ＰＴＡ－２１３４、ＰＴＡ－２１３５、ＰＴＡ－２１３６、ＰＴＡ－２１３７、ＰＴＡ－２１３８、ＰＴＡ－２１３９、ＰＴＡ－２１４０、ＰＴＡ－２１４１、ＰＴＡ－２１４２及びＰＴＡ－２１４３から成る群より選択された寄託番号の下で、２０００年６月２１日にＡＴＣＣに寄託された核酸分子である。

【００１６】

更なる側面では、本発明は、上文にて定義された抗体の重鎖及び／軽鎖をコードする核酸分子を含むベクターに関する。本発明は、また、そのような核酸で形質転換された細胞に関する。本発明は、更に、ハイブリドーマ細胞によって生産されたそのような核酸及び抗体で形質転換されたハイブリドーマ細胞株に関する。

より更なる側面では、本発明は、製薬的に許容可能な担体との混合物である、上文にて定義されているようなアゴニスト抗－ｔｒｋＣモノクローナル抗体の有効量を含んでなる製薬的な組成物に関する。

その他の側面では、本発明は、ここで開示されたアゴニスト抗－ｔｒｋＣ抗体の有効量を哺乳動物へ投与することを含んでなる、細胞変性に関わる疾患又は症状を治療する方法に関する。

【００１７】

更にその他の側面では、本発明は、神経障害又は神経変性疾患を治療する方法、又は、ここで開示のアゴニスト抗－ｔｒｋＣ抗体の有効量を哺乳動物へ投与することを含んでなる損傷神経細胞を修復する方法に関する。神経障害は、例えば、限定せずに、糖尿病性神経障害及び大線維状感覚（ｌａｒｇｅ ｆｉｂｅｒ ｓｅｎｓｏｒｙ）神経障害を含む抹消神経障害であり得る。神経変性は、例えば、筋萎縮性側索硬化症（ＡＬＳ）、アルツハイマー病、パーキンソン病、ハンチントン舞踏病であり得る。損傷ニューロンは、末梢、例えば、背根神経節ニューロン、運動ニューロン、例えば脊椎、又は中枢ニューロンからのニューロン等の感覚であってよく、そして、損傷は、トラウマ、神経毒へ曝すこと、代謝疾患、病原菌等を含む外及び内因子に起因し得る。

更なる側面では、本発明は、末梢ニューロンを本発明の抗体の有効量と接触させることを含む、末梢ニューロンの発達、増殖、維持又は再生を促進する方法に関する。

【００１８】

より更なる側面では、本発明は、哺乳動物被検体の細胞へのここにおける抗－ｔｒｋＣ抗体をコードする核酸を導入することによる、哺乳動物被検体の細胞変性が関与する疾患又は症状の治療（予防を含む）の方法に関する。この方法（遺伝子治療）は、好ましくは、神経障害又は神経変性疾患の治療、又は損傷神経細胞の回復に関する。従って、受容細胞は、好ましくは神経細胞である。

より更なる側面では、本発明は、遺伝子治療の用途に適した抗－ｔｒｋＣ抗体をコードする遺伝的物質（核酸）を含有する送達手段に関する。

付加的な側面では、本発明は、血管形成を誘導するために有効量の本発明の抗－ｔｒｋＣ抗体を送達することによって、血管形成を誘導する方法に関する。この送達は、特に、抗体の投与、及びこの抗体（例えば、遺伝子治療において）をコードする核酸の送達を含む。

【００１９】

更にその他の側面では、本発明は、配列番号：５８、配列番号：５９、配列番号：６０、配列番号：６１、配列番号：６２、配列番号：６３、配列番号：６４、配列番号：６５、配列番号：６６、配列番号：６７、配列番号：６８、配列番号：６９、配列番号：７０及び配列番号：７１から成る群より選択されたマウス又はヒト抗－ｔｒｋＣアゴニスト抗体重鎖又は軽鎖をコードする核酸分子に関する。本発明は、また、一つ又は複数の単離された核酸分子によってコードされたポリペプチドに関する。

その他の側面では、本発明は、マウス又はヒト抗－ｔｒｋＣアゴニスト抗体重鎖、軽鎖又は重及び軽鎖の双方をコードする核酸で形質転換した細胞全体に関する。

【００２０】
（好ましい実施態様の詳細な説明）
Ａ．定義
「ニューロトロフィン」という用語、及びその文法的異形は交換可能に使用され、神経成長因子（ＮＧＦ）及び連続的に関連した相同体を含むポリペプチドのファミリーを指す。ＮＧＦ、脳由来成長因子（ＢＤＮＦ、ａ．ｋ．ａ．　ＮＴ－２）、ニューロトロフィン－３（ＮＴ－３）、ニューロトロフィン－４及び－５（ＮＴ－４／５）、ニューロトロフィン－６（ＮＴ－６）、及びニューロトロフィン－７（ＮＴ－７）は、従来、このファミリーのメンバーとして同定された。
「ニューロトロフィン」という用語には、天然ソースから精製、組み換えＤＮＡ技術の方法によって調製、又は化学合成、又はこれら又はその他の方法の任意の組み合わせであろうとも、任意（ヒト又は非ヒト）の動物種の天然ニューロトロフィン、及びそれらの機能的誘導体を含む。「天然」又は「天然配列」ニューロトロフィンは、天然発生切断及び変異型、及び天然発生対立遺伝子変異体を含み、天然の任意のヒト又は非ヒト動物種に起こるニューロトロフィンのアミノ酸種を有する。
「ｔｒｋ」、「ｔｒｋポリペプチド」、「ｔｒｋレセプター」という用語、及びそれらの文法的異形は交換可能に使用され、少なくとも一つの天然ニューロトロフィンと結合することが可能なレセプターチロシンキナーゼスーパーファミリーのポリペプチドを指す。現在、同定されたこのファミリーのメンバーは、ｔｒｋＡ（ｐ１４０^{ｔｒｋＡ}）、ｔｒｋＢ、及びｔｒｋＣである。
【００２１】
ここで使用される場合の「細胞外ドメイン」又は「ＥＣＤ」という表現は、天然発生レセプターの細胞外ドメインのリガンド結合機能を共有する任意のポリペプチド配列を指す。細胞外ドメインのリガンド結合機能は、リガンドへ結合するポリペプチドの能力を指す。従って、より小さなセグメントがリガンド結合に十分であることが見出されたので、細胞外ドメインのすべてを含む必要はない。切断細胞外ドメインは、一般的には可溶性である。ＥＣＤという用語は、成熟レセプターの疎水性膜貫通ドメイン配列（及び、選択的には、膜貫通ドメインに対して１－２０アミノ酸Ｃ－末端及び／又はＮ－末端）が欠失されているポリペプチド配列を含む。
「アゴニスト抗－ｔｒｋＣ抗体」という用語は抗体を指し、それは、天然配列ｔｒｋＣレセプター及び／又はｔｒｋＣシグナリング機能によって媒介される下流の経路と結合及び活性化することができ、それによって特にＮＴ－３である、レセプターの天然リガンドの生物学的活性を模倣する。例えば、アゴニスト抗体は、ｔｒｋＣレセプターのＥＣＤドメインと結合し得るし、それによって、レセプターの二量体化が起こり、結果として細胞内触媒キナーゼドメインの活性化が生じる。結果として、これは、インビトロ及び／又はインビボでレセプターを発現する細胞の成長及び／又は分化を刺激し得ることとなる。本発明のアゴニスト抗体は、好ましくは、少なくともドメイン５の部分（アミノ酸位置約２６６から約３８１）及び／又はヒトｔｒｋＣレセプターのドメイン４（アミノ酸位置約１７８から約２６５）を含むエピトープ、又は、非ヒト、例えばマウスｔｒｋＣレセプターのエピトープを認識する。
【００２２】
本発明のアゴニスト抗－ｔｒｋＣ抗体との組み合わせで使用する場合、「生物学的活性」とは、ＮＴ－３と共通のエフェクター機能を有するｔｒｋＣの天然リガンドを指す。このエフェクター機能は、好ましくは、ｔｒｋＣレセプターチロシンキナーゼ及び／又はｔｒｋＣシグナリング機能によって媒介される下流の経路と結合及び活性化する能力である。限定なしに、好ましい生物学的活性には、特にニューロンのインビトロ又はインビボでの損傷細胞の発達、増殖、維持及び／又は再生を促進する能力が含まれ、末梢（交換神経、副交感神経、感覚、及び腸）ニューロン、運動ニューロン、及び中枢（脳及び脊髄）ニューロン、及び非ニューロン性細胞、例えば末梢血白血球が含まれる。特に好ましい生物学的活性は、神経障害、例えば、末梢神経障害又は他の神経変性疾患、又は損傷神経細胞の

修復を治療（予防を含む）する能力である。損傷ニューロンは、感覚、交感神経、副交感神経、又は腸、例えば背根神経節、運動ニューロン、及び中枢神経、例えば脊髄からのニューロンであり得、そして、損傷は、トラウマ、手術、発作、虚血、感染、代謝疾患、栄養失調、及び種々の悪性腫瘍を含む、任意の原因であり得る。その他の特定の生物学的活性は、血管形成を誘導する能力である。

【００２３】

ここで用いられているように、「治療」とは、有益又は望ましい臨床的結果を得るための手段である。この発明の目的としては、限定されるものではないが、有益又は望ましい臨床的結果には、検出可能又は検出不可能に関わらず、症状の緩和、疾患の程度を小さくすること、疾患の安定した状態（すなわち、悪化させない）、疾患の進展を遅らせる又は遅くすること、疾患の状態を改善又は緩和すること、そして緩解（部分又は全体）が含まれる。「治療」とは、また、治療を受けていない場合に期待される生存に比べて生存を引き延ばすことを意味する。「治療」とは、疾病の病状の発展を防ぐ狙い又は改善することで実行される介入である。従って、「治療」とは、治療上の処置及び予防的又は再発防止の双方を指す。治療が必要される者には、疾患が予防されるべき者と同時に疾患をすでに患っている者を含む。特に、治療は、例えば神経細胞の病状のような、細胞変性の損傷の病状を直接に防ぎ、鈍化させるか、さもなくば減少させ得るか、又は、ニューロンを他の治療剤による治療に対してより敏感するなどを細胞へ与える。好ましい実施態様では、治療は、標的ニューロンの機能の衰退を減じるか鈍化させ、及び／又回復を刺激する。

【００２４】

（慢性の）神経変性疾患又は慢性神経系傷害の「病状」には、制限なく、神経性機能障害、変性、損傷及び／又は死を含む、患者の健康に影響をする全ての現象が含まれる。

「神経変性疾病」及び「神経変性疾患」という用語は、制限なく、末梢神経障害；運動ニューロン疾患、例えば筋萎縮性側索硬化症（ＡＬＳ、ルー・ゲーリック病）、顔面麻痺、及び脊髄筋萎縮症又は麻痺を含む種々の症状；及び他のヒト神経変性疾患、例えばアルツハイマー病、パーキンソン病、てんかん、多発性硬化症、ハンチントン舞踏病、ダウン症、神経性難聴、及びメニエール病を含む神経性変性及び／又は機能障害を含むすべての疾患の症状を含め、広い意味で用いられる。

【００２５】

「末梢神経障害」は、末梢神経系に影響を与える疾患であり、運動、感覚、運動感覚、又は自律神経の１つ又は複数の機能障害として現れることが多い。例えば、末梢神経障害は、遺伝子的に獲得され、全身性疾患によりもたらされ、又は毒剤、例えば神経毒薬、例えば抗悪性腫瘍薬、又は産業性又は環境性汚染物質によって誘発される可能性がある。「末梢感覚神経障害」は、末梢感覚ニューロンの変性によって特徴付けられ、それは特発性であり得、例えば、糖尿病（糖尿病性ニューロパシー）、癌における抗悪性腫瘍薬治療（例えば、ビンクリスチン、シスプラチン、メトトレキサート、３’－アジド－３’－デオキシチミジン、又はタキサン、例えばパクリタキセル［ＴＡＸＯＬ（登録商標）、ブリストル・マイヤーズスクイブ腫瘍学、プリンストン、ニュージャージー］及びドキシタキセル［ＴＡＸＯＴＥＲＥ（登録商標）、ローヌプーランク、アントニー，フランス］の化学療法剤による治療）、アルコール依存症、エイズ，後天性免疫不全症候群（ＡＩＤＳ）、又は遺伝子的疾病素質の結果として起こり得る。遺伝的後天性抹消神経障害には、例えば、レフスム病、クラッベ病、異染性ロイコジストロフィー、ファブリー病、デジェリン・ソッタ病、無βリポタンパク質血症、及びシャルコー・マリー・ツース（ＣＭＴ）病（腓骨筋萎縮症又は遺伝性運動感覚性ニューロパチシー（ＨＭＳＮ）としても知られている）。多くの型の抹消神経障害は、数ヶ月又は数年の過程でゆっくりと進行する。臨床診療では、そのような神経障害は慢性だといわれている。時折、末梢神経障害は、数日の過程を通して急速に進行し、急性と呼ばれている。末梢神経障害は、通常、感覚及び運動神経をともに冒して混合性の感覚及び運動神経障害を起こすが、単なる感覚及び単なる運動神経障害も知られている。

【００２６】

この発明の文脈で用いられているような「毒剤」という用語は、その化学作用を通して神経系の成分の作用を損傷し、弱め、又は阻害する物質を指すことを意味する。毒剤の長い一覧表（「神経毒剤」とも呼ばれている）には、限定されることなく、化学療法剤、例えば上に記載されているアルコール、金属、産業性毒素、食品及び医薬品の汚染物質などが含まれる。

治療の目的の「哺乳動物」とは、ヒト、家畜、及び動物園，又はペット動物、例えば犬、馬、ネコ等を含む哺乳動物として分類される任意の動物を指す。このましくは哺乳動物とはヒトである。

【００２７】

「ｔｒｋＣイムノアドヘシン」という用語は、「ｔｒｋＣ－イムノグロブリンキメラ」という表現と交換可能であり、ｔｒｋＣの一分部（一般には、その細胞外ドメイン）を免疫グロブリン配列と結合させるキメラ分子を指す。必ずしもそうではないが、免疫グロブリン配列は、このましくは免疫グロブリン定常ドメインである。適切な免疫グロブリン定常ドメイン（免疫アドヘシン）と結合しているレセプター配列から構築されたキメラは、当該分野で知られている。文献にに報告されている免疫アドヘシンには、Ｔ細胞レセプター*（Ｇａｓｃｏｉｇｎｅら．，Ｐｒｏｃ．Ｎａｔｌ．Ａｃａｄ．Ｓｃｉ．ＵＳＡ，８４：２９３６－２９４０［１９８７］）；ＣＤ４*（Ｃａｐｏｎら．，Ｎａｔｕｒｅ ３３７：５２５－５３１［１９８９］；Ｔｒａｕｎｅｋｅｒら．，Ｎａｔｕｒｅ，３３９：６８－７０［１９８９］；Ｚｅｔｔｍｅｉｓｓｌら．，ＤＮＡ Ｃｅｌｌ Ｂｉｏｌ．９：３４７－３５３［１９９０］；Ｂｙｍら．，Ｎａｔｕｒｅ，３４４：６６７－６７０［１９９０］；Ｌ－ｓｅｌｅｃｔｉｎ（ｈｏｍｉｎｇ ｒｅｃｅｐｔｏｒ）（Ｗａｔｓｏｎら．，Ｊ．Ｃｅｌｌ．Ｂｉｏｌ．，１１０：２２２１－２２２９［１９９０］；Ｗａｔｓｏｎら．，Ｎａｔｕｒｅ，３４９：１６４－１６７［１９９１］）；ＣＤ４４*（Ａｒｕｆｆｏら．，Ｃｅｌｌ，６１：１３０３－１３１３［１９９０］）；ＣＤ２８*及びＢ７*（Ｌｉｎｓｌｅｙら．，Ｊ．Ｅｘｐ．Ｍｅｄ．，１７３：７２１－７３０［１９９１］）；ＣＴＬＡ－４*（Ｌｉｓｌｅｙら．，Ｊ．Ｅｘｐ．Ｍｅｄ．１７４：５６１－５６９［１９９１］）；ＣＤ２２*（Ｓｔａｍｅｎｋｏｖｉｃら．，Ｃｅｌｌ，６６：１１３３－１１１４４［１９９１］）；ＴＮＦレセプター（Ａｓｈｋｅｎａｚｉら．，Ｐｒｏｃ．Ｎａｔｌ．Ａｃａｄ．Ｓｃｉ．ＵＳＡ，８８：１０５３５－１０５３９［１９９１］；Ｌｅｓｓｌａｕｅｒら．，Ｅｕｒ．Ｊ．Ｉｍｍｕｎｏｌ．，２７：２８８３－２８８６［１９９１］；Ｐｅｐｐｅｌら．，Ｊ．Ｅｘｐ．Ｍｅｄ．，１７４：１４８３－１４８９［１９９１］；ＮＰレセプター（Ｂｅｎｎｅｔｔら．，Ｊ．Ｂｉｏｌ．Ｃｈｅｍ．２６６：２３０６０－２３０６７［１９９１］；及びＩｇＥレセプターα*（Ｒｉｄｇｗａｙら．，Ｊ．Ｃｅｌｌ．Ｂｉｏｌ．，１１５：ａｂｓｔｒ．１４４８［１９９１］）の融合物が含まれ、星印（*）は、レセプターが免疫グロブリンスーパーファミリーのメンバーであることを示している。

【００２８】

本発明の文脈における「単離された」核酸又はポリペプチドとは、核酸又はポリペプチドの動物又はヒトに存在する夾雑核酸又はポリペプチドから同定及び単離された核酸又はポリペプチドである。この核酸又はポリペプチドは、更に下記にて記載し定義したような診断アッセイの考察を用いて診断又はプローブの目的のために標識され得る。

一般的に、「アミノ酸配列変異体」という用語は、参考（例えば天然配列）のポリペプチドと比較してアミノ酸配列に幾つかの違いがある分子を指す。アミノ酸の改変は、置換、挿入、欠損又は天然アミノ酸配列におけるそのような変化の任意の所望される組み合わせであってもよい。

【００２９】

「ＤＮＡ配列コード化」、「ＤＮＡコード化」及び「核酸コード化」とは、デオキシリボ核酸のストランドに沿ったデオキシリボヌクレオチドの順序又は配列を指す。これらのデオキシリボヌクレオチドの順序は、ポリペプチド鎖に沿ったアミノ酸の順序を確定する。

従って、ＤＮＡ配列は、アミノ酸配列をコードする。
「複製可能な発現ベクター」及び「発現ベクター」という用語は、ＤＮＡの断片、通常は外来ＤＮＡの断片をそれに挿入させたであろう二重鎖を指す。外来ＤＮＡは、異種ＤＮＡとして定義される、天然には宿主細胞では見出されないＤＮＡである。ベクターは、外来又は異種ＤＮＡを適切な宿主細胞へ送達するために使用される。一度宿主細胞へ送達されると、ベクターは宿主染色ＤＮＡとは独立して複製することができ、そのベクターの幾つかのコピーとその挿入（外来）ＤＮＡが生成され得る。更には、このベクターは、外来ＤＮＡをポリペプチドへ翻訳することを可能にするのに必要なエレメントを含む。従って、外来ＤＮＡによってコードされているポリペプチドの多くの分子が迅速に合成できる。
【００３０】
「コントロール配列」という用語は、特定の宿主生物での作用可能に結合したコード化配列の発現に必要なＤＮＡ配列を指す。例えば、原核生物に適したコントロール配列には、プロモーター、選択的にオペレーター配列、リボゾーム結合部位、そして恐らくは、まだよく理解されていない配列のような他のものが含まれる。真核生物は、プロモーター、ポリアデニル化シグナル、そしてエンハンサーを利用することが知られている。
核酸は、その他の核酸と機能的な関係で置換されると「作用可能に結合する」。例えば、シグナルペプチド又は分泌リーダーに関するＤＮＡは、ポリペプチドの分泌に加わってプレタンパク質として発現されると、ポリペプチドに関するＤＮＡと作用可能に結合する；プロモーター又はエンハンサーは、配列の転写に作用するならば、コード化配列と作用可能に結合する；又はリボゾーム結合部位は、翻訳を促進するように配置されると、コード化配列と作用可能に結合する。一般的に、「作用可能に結合」とは、結合しているＤＮＡ配列は近接しており、分泌リーダーの場合に近接し、リーディング・フェーズである。しかしながら、エンハンサーは近接している必要がない。結合は、好都合な制限部位でのライゲーションによって完遂される。そのような部位が存在しないならば、次いで合成オリゴヌクレオチドアダプター又はリンカーは、従来のやり方に従って使用される。
【００３１】
本発明の文脈では、「細胞」、「細胞株」、及び「細胞培養」という表現は交換可能に用いられ、全てのそのような表示には子孫が含まれる。従って、「形質転換体」及び「形質転換（宿主）細胞」という語句には、トランスファーの数に関わらず、それから誘導された一次被験細胞及び培養を含む。また、故意又は不慮の変異のために、全ての子孫がＤＮＡ内容物について正確には同一でないことがあり得る。当初の形質転換細胞においてスクリーニングされたのと同じ機能又は生物活性を有する変異子孫が含まれる。識別可能な表示が意図された場合、それは状況から明かである。
【００３２】
「外因性」エレメントは、ここでは、細胞にとって外来性のもの、又はエレメントが通常は見出されない宿主細胞核酸内に位置するが細胞にとって相同的なものである核酸配列を意味する。
「抗体」（Ａｂｓ）及び「免疫グロブリン」（Ｉｇｓ）は同じ構造的特徴を持つ糖タンパク質である。抗体は特定の抗原に対する結合特異性を示すが、免疫グロブリンは抗体及び抗原特異性を持たない他の抗体様分子の両方を含む。後者の種類のポリペプチドは、例えば、リンパ系では低レベルで産生され、ミエローマにおいて産生レベルが増加する。
「天然抗体」及び「天然免疫グロブリン」は、通常、２つの同一の軽（Ｌ）鎖及び２つの同一の重（Ｈ）鎖からなる、約１５０，０００ダルトンの異種四量体糖タンパク質である。各軽鎖は一つの共有ジスルフィド結合により重鎖に結合しており、異なる免疫グロブリンアイソタイプ重鎖中のジスルフィド結合の数は相違する。また各重鎖と軽鎖は、規則的に離間した部位との間で鎖内ジスルフィド架橋を有している。各重鎖は、多くの定常ドメインに続いて可変ドメイン（V_H）を一端に有する。各軽鎖は、一端に可変ドメイン（V_L）を、他端に定常ドメインを有し；軽鎖の定常ドメインは重鎖の第一定常ドメインと整列し、軽鎖の可変ドメインは重鎖の可変ドメインと整列している。特定のアミノ酸残基が、軽鎖及び重鎖可変ドメイン間の界面を形成すると考えられている。

【００３３】
「可変」という用語は、可変ドメインのある部位が、抗体の中で配列が広範囲に異なるという事を意味し、その特定の抗原に対する各特定の抗体の結合及び特異性に利用される。しかしながら、可変性は抗体の可変ドメインにわたって一様には分布していない。軽鎖及び重鎖の可変ドメインの両方の高頻度可変領域と呼ばれる３つのセグメントに濃縮される。可変ドメインのより高度に保存された部分はフレームワーク領域（ＦＲ）と呼ばれる。天然の重鎖及び軽鎖の可変ドメインは、ループ結合を形成し、そしてある場合にはその一部を形成するβ－シート構造を形成する、３つの高頻度可変領域によって結合しているシート立体配置を主としてとっている４つのＦＲ（それぞれＦＲ１，ＦＲ２，ＦＲ３及びＦＲ４）を構成する。各鎖の高度可変領域は、ＦＲによって近接してまとまっていて、他の鎖の高度可変領域とともに、抗体の抗原結合部位の形成に寄与する（Ｋａｂａｔら．，Ｓｅｑｕｅｎｃｅｓ ｏｆ Ｐｒｏｔｅｉｎｓ ｏｆ Ｉｍｍｕｎｏｌｏｇｉｃａｌ Ｉｎｔｅｒｅｓｔ，５ｔｈ Ｅｄ．Ｐｕｂｌｉｃ Ｈｅａｌｔｈ Ｓｅｒｖｉｃｅ，アメリカ国立衛生研究所，ベセズダ，メリーランド（１９９１），６４７－６６９項）。定常ドメインは、抗体を抗原に結合させることに直接は関与していないが、例えば抗体の抗体依存細胞毒性への関与などの種々のエフェクター機能を示す。

【００３４】
ここで用いられる「高度可変領域」という用語は、抗原結合を担う抗体のアミノ酸残基を指す。高度可変領域は、「相補性決定領域」又は「ＣＤＲ」のアミノ酸残基（すなわち、軽鎖可変領域の残基２４－３４（Ｌ１）、５０－５６（Ｌ２）及び８９－９７（Ｌ３）及び重鎖可変領域の３１－３５（Ｈ１）、５０－６５（Ｈ２）及び９５－１０２（Ｈ３）；Ｋａｂａｔら．，Ｓｅｑｕｅｎｃｅｓ ｏｆ Ｐｒｏｔｅｉｎｓ ｏｆ Ｉｍｍｕｎｏｌｏｇｉｃａｌ Ｉｎｔｅｒｅｓｔ，５ｔｈ Ｅｄ．Ｐｕｂｌｉｃ Ｈｅａｌｔｈ Ｓｅｒｖｉｃｅ，アメリカ国立衛生研究所，ベセズダ，メリーランド（１９９１））及び／又は「高度可変ループ」のそれら残基（すなわち、軽鎖可変ドメインの残基２６－３２（Ｌ１）、５０－５２（Ｌ２）及び９１－９６（Ｌ３）及び重鎖可変ドメインの２６－３２（Ｈ１）、５３－５５（Ｈ２）及び９６－１０１（Ｈ３）；Ｃｈｏｔｈｉａ及びＬｅｓｋ Ｊ．Ｍｏｌ．Ｂｉｏｌ．１９６：９０１－９１７（１９８７））を含む。「フレームワーク」又は「ＦＲ」残基は、ここで定義されているような高度可変領域残基以外の可変ドメイン残基である。

抗体のパパイン消化は、それぞれが単一の抗原結合部位を有する「Ｆａｂ」断片、及びその名前が結晶化が容易であることを示している残基「Ｆｃ」断片と呼ばれる二つの抗原結合断片を生産する。ペプシン処理によって、二つの抗原結合部位を有し、さらに架橋抗原が可能なＦ（ａｂ’）$_2$ 断片が生じる。

【００３５】
「Ｆｖ」は、完全な抗原認識及び結合部位を含む最小抗体断片である。この領域は、堅く非共有的に結合した１つの重鎖と１つの軽鎖の可変ドメインの二量体からなる。この配置では、V_H－V_L 二量体の表面における抗原結合部位を規定するために各可変領域の３つの高度可変領域が相互作用する。正確には、６つの高度可変領域が抗体に抗原結合特異性を与える。しかし、単一の可変ドメイン（又は抗原特異的な３つのＣＤＲしか含まないＦｖの半分）でさえも抗原を認識し結合する能力を持つが、結合部位全体よりは親和性が低い。

また、Ｆａｂ断片は軽鎖の定常ドメイン及び重鎖の第１の定常ドメイン（ＣＨ１）も含む。Ｆａｂ’断片は、抗体ヒンジ領域からの１つ又は複数のシステインを含む重鎖ＣＨ１ドメインのカルボキシル末端における数個の残基の付加によりＦａｂ断片と相違する。Ｆａｂ’－ＳＨは、ここにおいて、定常ドメインのシステイン残基が遊離のチオール基を持つＦａｂ’の記号である。Ｆ（ａｂ’）$_2$ 抗体断片は、元々、それらの間にヒンジシステインを持つＦａｂ’断片の対として生成された。抗体断片の他の化学的結合も知られている。

【００３６】

任意の脊椎動物種からの抗体（免疫グロブリン）の「軽鎖」は、それらの定常ドメインのアミノ酸配列に基づいて、カッパ（κ）及びラムダ（λ）と呼ばれる１つ又は２つの明らかに異なる型に分類できる。
それらの重鎖の定常ドメインのアミノ酸配列に基づいて、免疫グロブリンは異なるクラスに分けられる。免疫グロブリンの５つの主要なクラス：ＩｇＡ、ＩｇＤ、ＩｇＥ、ＩｇＧ、及びＩｇＭがあり、これらの幾つかは、更にサブクラス（アイソタイプ）、例えばＩｇＧ１、ＩｇＧ２、ＩｇＧ３、ＩｇＧ４、ＩｇＡ及びＩｇＡ２に分けられる。異なるクラスの免疫グロブリンと一致する重鎖定常ドメインは、，，，，及び，とそれぞれ呼ばれている。免疫グロブリンのサブユニット構造及び異なるクラスの三次元構造は、良く知られている。

【００３７】
「抗体」という用語は最も広い意味において使用され、特にヒト、非ヒト（例えばマウス）及びヒト化モノクローナル抗体（全長モノクローナル抗体を含む）、ポリクローナル抗体、多重特異性抗体（例えば二重特異性抗体）、及び所望の生物活性を示す限りは抗体断片を包含する。
「抗体断片」は全長抗体の一部、一般にはその抗原結合又は可変領域を含む。抗体断片の例には、Ｆａｂ、Ｆａｂ’、Ｆ（ａｂ’）$_2$及びＦｖ断片；線状抗体；ダイアボディ；単鎖抗体分子；及び抗体断片から生じた多重特異性抗体が含まれる。

【００３８】
ここで使用される「モノクローナル抗体」という用語は、実質的に均一な抗体の集団から得られる抗体を指す、すなわち、集団を構成する個々の抗体が、少量で存在しうる自然に生じる可能な突然変異を除いて同一である。モノクローナル抗体は高度に特異的であり、一つの抗原部位に対応する。更に、異なる決定基（エピトープ）に対応する異なる抗体を典型的には含む通常の（ポリクローナル）抗体とは異なり、各モノクローナル抗体は抗原の単一の決定基に対応する。「モノクローナル」との形容は、実質的に均一な抗体集団から得られたという抗体の性質を示し、抗体を何か特定の方法で生産しなければならないことを意味するものではない。例えば、本発明において使用されるモノクローナル抗体は、Ｋｏｈｌｅｒらによって最初にＮａｔｕｒｅ ２５６：４９５ （１９７５）に記載されたハイブリドーマ法によって作ることができ、あるいは組換えＤＮＡ法（例えば米国特許第４，８１６，５６７号を参照）によって作ることができる。「モノクローナル抗体」は、例えばＣｌａｃｋｓｏｎら，Ｎａｔｕｒｅ ３５２：６２４－６２８ （１９９１） 及びＭａｒｋｓら，Ｊ．Ｍｏｌ．Ｂｉｏｌ． ２２２：５８１－５９７ （１９９１）に記載された技術を用いてファージ抗体ライブラリから単離してもよい。

【００３９】
ここでもモノクローナル抗体は特に、重鎖及び／又は軽鎖の一部が特定の種から由来するか特定の抗体のクラス又はサブクラスに属する抗体中の対応する配列と同一もしくは相同である一方、鎖の残りが他の種から由来するか他の抗体のクラス又はサブクラスに属する抗体中の対応する配列と同一もしくは相同である「キメラ」抗体（免疫グロブリン）、並びに、それが所望の生物活性を示す限り、そのような抗体の断片を含む（米国特許第４，８１６，５６７号；及びＭｏｒｒｉｓｏｎら，Ｐｒｏｃ．Ｎａｔｌ．Ａｃａｄ．Ｓｃｉ．ＵＳＡ ８１：６８５１－６８５５（１９８４））。

【００４０】
非ヒト（例えばマウス）抗体の「ヒト化」型は、非ヒト免疫グロブリンから誘導された最小配列を含むキメラ抗体である。大抵は、ヒト化抗体は、レシピエントの高頻度可変領域由来の残基が、所望の特異性、親和性及び能力を有するマウス、ラット、ウサギ又は非ヒト霊長類のようなヒト以外の種（ドナー抗体）の高頻度可変領域由来の残基により置き換えられている、ヒト免疫グロブリン（レシピエント抗体）である。幾つかの例では、ヒト免疫グロブリンのＦＲ残基が、対応する非ヒト残基に置き換えられている。更に、ヒト化抗体は、レシピエント抗体にもドナー抗体にも見いだされない残基を含んでいてもよい。これらの修飾は、抗体の挙動を更に洗練するためになされる。一般に、ヒト化抗体は、少

なくとも１個、典型的には２個の可変ドメインの実質的に全てを含み、ここで、全て又は実質的に全ての高頻度可変領域は非ヒト免疫グロブリンのものに対応し、全て又は実質的に全てのＦＲ領域はヒト免疫グロブリンのものである。ヒト化抗体は、場合によっては、免疫グロブリン定常部（Ｆｃ）、典型的にはヒト免疫グロブリンの定常部の一部を少なくとも含むであろう。更になる詳細については、Ｊｏｎｅｓら，Ｎａｔｕｒｅ３２１：５２２－５２５（１９８６）；Ｒｉｅｃｈｍａｎｎら，Ｎａｔｕｒｅ３３２：３２３－３２９（１９８８）；及びＰｒｅｓｔａ，Ｃｕｒｒ．Ｏｐ．Ｓｔｒｕｃｔ．Ｂｉｏｌ．２：５９３－５９６（１９９２）を参照されたい。

【００４１】

「単鎖Ｆｖ」又は「ｓＦｖ」抗体断片は抗体のV_H及びV_Lドメインを含に、ここでこれらドメインはポリペプチド単鎖中に存在する。一般にＦｖポリペプチドは、ｓＦｖをして抗原結合に対する所望の構造を形成せしめるポリペプチドリンカーをV_HドメインとV_Lドメイン間に更に含む。ｓＦｖのレビューについては、Ｐｌｕｃｋｔｈｕｎ，Ｔｈｅ Ｐｈａｒｍａｃｏｌｏｇｙ ｏｆ Ｍｏｎｏｃｌｏｎａｌ Ａｎｔｉｂｏｄｉｅｓ，ｖｏｌ．１１３，Ｒｏｓｅｎｂｕｒｇ及びＭｏｏｒｅ編，Ｓｐｒｉｎｇｅｒ－Ｖｅｒｌａｇ，Ｎｅｗ Ｙｏｒｋ，２６９－３１５頁（１９９４）を参照されたい。

「ダイアボディ」なる用語は、二つの抗原結合部位を持つ小さい抗体断片を指し、その断片は同一のポリペプチド鎖（V_H－V_L）内で軽鎖可変ドメイン（V_L）に重鎖可変ドメイン（V_H）が結合している。非常に短いために同一鎖上で二つのドメインの対形成を可能にするリンカーを使用して、ドメインを他の鎖の相補ドメインと強制的に対形成させ、二つの抗原結合部位を創製する。ダイアボディーは、例えば、ＥＰ４０４，０９７；国際公開９３／１１１６１；及びＨｏｌｌｉｎｇｅｒら，Ｐｒｏｃ．Ｎａｔｌ．Ａｃａｄ．Ｓｃｉ．ＵＳＡ ９０：６４４４－６４４８（１９９３）に更に詳細に記載されている。

【００４２】

本出願において使用する場合、「線状抗体」なる表現は、Ｚａｐａｔａら，ＰｒｏｔｅｉｎＥｎｇ．８（１０）：１０５７－１０６２（１９９５）に記載された抗体を指す。簡単に言えば、これらの抗体は一対の抗原結合部位を形成する一対のタンデムＦｄセグメント（V_H－C_H1－V_H－C_H1）を含む。

「エピトープ」という用語は、抗原決定基として、抗体と結合することができる抗体又はその一部分を意味する。

【００４３】

天然配列ヒトｔｒｋＣのアミノ酸配列内のドメイン５及び／又は４と、天然配列非ヒトｔｒｋＣレセプターの同等のエピトープと結合する抗体は、「エピトープマッピング」によって同定される。タンパク質上のエピトープの位置をマッピング及び特徴付ける多くの方法が当該分野で知られており、これには、例えばＨａｒｌｏｗ及びＬａｎｅ，Ｕｓｉｎｇ Ａｎｔｉｂｏｄｉｅｓ，ａ Ｌａｂｏｒａｔｏｒｙ Ｍａｎｕａｌ，Ｃｏｌｄ Ｓｐｒｉｎｇ Ｈａｒｂｏｒ Ｌａｂｏｒａｔｏｒｙ Ｐｒｅｓｓ，コールド・スプリング・ハーバー，ニューヨーク，１９９９の第１１章に記載されているような抗体－抗原複合体の結晶構造を解析すること、競合アッセイ、遺伝子断片発現アッセイ、及び合成ペプチドベースアッセイが含まれる。競合ＥＬＩＳＡアッセイは、具体的に実施例１に記載されている。遺伝子断片発現アッセイによると、タンパク質をコードしているオープンリーディングフレームは、ランダムに又は特定の遺伝的構築物のいずれかによって断片化されていて、タンパク質の発現断片の試験される抗体との活性が測定される。例えば、遺伝子断片はＰＣＲによって生産され、次いで、放射性アミノ酸の存在下で転写されてインビトロでタンパク質へ翻訳される。抗体の放射標識タンパク質断片への結合は、次いで免疫沈降とゲル電気泳動によって測定される。特定のエピトープは、また、ファージ粒子の表面に表示されたランダムペプチド配列の大きなライブラリ（ファージライブラリ）を用いることによって同定される。或いは、重なり合ったペプチド断片の特定されたライブラリは、単純な結合アッセイで、試験抗体との結合を試験することができる。後者の方法は、約５から１

５のアミノ酸の直鎖状エピトープを特定するために適している。

【００４４】

二つの抗体が同一又は立体的に重なり合ったエピトープを認識する場合、抗体は、参考の抗体のように、「本質的に同じエピトープ」と結合する。二つのエピトープが同一又は立体的に重なり合ったエピトープと結合するかどうかを決定するために、最も広くそして迅速な方法は競合アッセイであり、それは、標識抗原又は標識抗体のいずれかを用いて、全ての異なる形式で構成することができる。通常は、抗原は９６ウェルプレート上に固定化され、標識抗体の結合を阻止する非標識抗体の能力は、放射性又は酵素標識を用いて測定する。競合ＥＬＩＳＡアッセイは、実施例１に開示されている。

ここで用いられる場合のアミノ酸又はアミノ酸残基という用語は、変異体について以下でさらに説明するように、天然発生Ｌアミノ酸又はＤアミノ酸を意味する。共通して使用される一及び三文字略語が、アミノ酸についてここで用いられる（Ｂｒｕｃｅ Ａｌｂｅｒｔｓら，Ｍｏｌｅｃｕｌａｒ Ｂｉｏｌｏｇｙ ｏｆ ｔｈｅ Ｃｅｌｌ，Ｇａｒｌａｎｄ Ｐｕｂｌｉｓｈｉｎｇ，Ｉｎｃ．，Ｎｅｗ Ｙｏｒｋ（３ｄ ｅｄ．１９９４））。

【００４５】

ハイブリダイゼーションは、（１）洗浄のために低イオン強度及び高温度、例えば、５０℃において０．０１５Ｍの塩化ナトリウム／０．００１５Ｍのクエン酸ナトリウム／０．１％のドデシル硫酸ナトリウムを用いる、又は（２）ハイブリダイゼーション中にホルムアミド等の変性剤、例えば、４２℃において５０％（ｖｏｌ／ｖｏｌ）ホルムアミドと０．１％ウシ血清アルブミン／０．１％フィコール／０．１％のポリビニルピロリドン／５０ｍＭのｐＨ６．５のリン酸ナトリウムバッファー、及び７５０ｍＭの塩化ナトリウム、７５ｍＭクエン酸ナトリウムを用いることを意味する「ストリンジェントな条件」の下で好んで行われる。その他の例としては、５０％ホルムアミド、５ｘＳＳＣ（０．７５ＭのＮａＣｌ、０．０７５Ｍのクエン酸ナトリウム）、５０ｍＭのリン酸ナトリウム（ｐＨ６／８）、０．１％のピロリン酸ナトリウム、５ｘデンハード液、超音波処理サケ精子ＤＮＡ（５０ｇ／ｍｌ）、０．１％ＳＤＳ、及び１０％のデキストラン硫酸を４２℃で用い、４２℃で０．２ｘＳＳＣ及び０．１％ＳＤＳで洗浄することである。

【００４６】

Ｂ．本発明を実行する方法

本発明は、アゴニストヒト及び非ヒトモノクローナル抗体（後者のヒト化型を含む）に関し、それらはＮＴ－３のある生物学的特性、ｔｒｋＣレセプターの天然リガンドを模倣する。マウス及びヒト抗－ｔｒｋＣ抗体の生産に関する一般的な技術は、当該分野で知られており、下記にて記載されている。アゴニスト抗体の選択を含むさらなる詳細は、実施例１に示されている。

【００４７】

１．抗体の調製

（ｉ）ポリクローナル抗体

ポリクローナル抗体を調製する方法は、当該分野で知られている。例えば、ポリクローナル抗体は、免疫剤の一つ又は複数回、そして所望するならばアジュバンドの注入によって哺乳動物で産生することができる。免疫剤を免疫化した哺乳動物にとって免疫原性であることが知られているタンパク質、例えば血清アルブミン、又は大豆トリプシンインヒビターと結合させることが有益であり得る。用いられ得るアジュバンドの例には、完全フロインドアジュバント及びＭＰＬ－ＴＤＭが含まれる。

【００４８】

（ｉｉ）モノクローナル抗体

モノクローナル抗体は、Ｋｏｈｌｅｒら，Ｎａｔｕｒｅ，２５６：４９５（１９７５）により最初に記載されたハイブリドーマ法を用いて作製でき、又は組換えＤＮＡ法（米国特許第４，８１６，５６７号）によって作成することができる。

ハイブリドーマ法においては、マウス又はその他の適当な宿主動物、例えばハムスターもしくはマカクザルを上記のように免疫し、免疫化に用いられたタンパク質と特異的に結合

する抗体を産生する、又は産生することのできるリンパ球を導き出す。別法として、リンパ球をインビトロで免疫することもできる。次に、リンパ球を、ポリエチレングリコールのような適当な融合剤を用いて骨髄腫細胞と融合させ、ハイブリドーマ細胞を形成させる（Ｇｏｄｉｎｇ，Ｍｏｎｏｃｌｏｎａｌ Ａｎｔｉｂｏｄｉｅｓ：Ｐｒｉｎｃｉｐｌｅｓ ａｎｄ Ｐｒａｃｔｉｃｅ，５９－１０３頁（Ａｃａｄｅｍｉｃ Ｐｒｅｓｓ，１９８６））。

このようにして調製されたハイブリドーマ細胞を、融合していない親の骨髄腫細胞の増殖または生存を阻害する一又は複数の物質を好ましくは含む適当な培地に蒔き、増殖させる。例えば、親の骨髄腫細胞が酵素ヒポキサンチングアニジンホスホリボシルトランスフェラーゼ（ＨＧＰＲＴ又はＨＰＲＴ）を欠失するならば、ハイブリドーマのための培地は、典型的には、ＨＧＰＲＴ－欠失細胞の増殖を妨げる物質であるヒポキサンチン、アミノプテリン、及びチミジンを含有するであろう（ＨＡＴ培地）。

【００４９】

好ましい骨髄腫細胞は、効率的に融合し、選択された抗体産生細胞による抗体の安定な高レベルの発現を支援し、ＨＡＴ培地のような培地に対して感受性である細胞である。これらの中でも、好ましい骨髄腫株化細胞は、マウス骨髄腫ライン、例えば、ソーク・インスティテュート・セル・ディストリビューション・センター、サンディエゴ、カリフォルニア、ＵＳＡより入手し得るＭＯＰ－２１およびＭ．Ｃ．－１１マウス腫瘍、及び、アメリカン・タイプ・カルチャー・コレクション、ロックヴィル、メリーランド、ＵＳＡより入手し得るＳＰ－２又はＸ６３－Ａｇ８－６５３細胞から誘導されるものである。ヒト骨髄腫及びマウス－ヒトヘテロ骨髄腫株化細胞もまたヒトモノクローナル抗体の産生のために開示されている（Ｋｏｚｂｏｒ，Ｊ．Ｉｍｍｕｎｏｌ．，１３３：３００１（１９８４）；Ｂｒｏｄｅｕｒら，Ｍｏｎｏｃｌｏｎａｌ Ａｎｔｉｂｏｄｙ Ｐｒｏｄｕｃｔｉｏｎ Ｔｅｃｈｎｉｑｕｅｓ ａｎｄ Ａｐｐｌｉｃａｔｉｏｎｓ，５１－６３頁、（Ｍａｒｃｅｌ Ｄｅｋｋｅｒ，Ｉｎｃ．，Ｎｅｗ Ｙｏｒｋ，１９８７））。

ハイブリドーマ細胞が生育している培地を、抗原に対するモノクローナル抗体の産生について検定する。好ましくは、ハイブリドーマ細胞により産生されるモノクローナル抗体の結合特異性は、免疫沈降又はインビトロ結合検定、例えばラジオイムノアッセイ（ＲＩＡ）又は酵素結合免疫吸着検定（ＥＬＩＳＡ）によって測定する。

【００５０】

例えば、モノクローナル抗体の結合親和性は、Ｍｕｎｓｏｎら．，Ａｎａｌ．Ｂｉｏｃｈｅｍ．，１０７：２２０（１９８０）のスキャッチャード分析によって測定することができる。

所望の特異性、親和性、及び／又は活性の抗体を産生するハイブリドーマ細胞が確定された後、該クローンを限界希釈法によりサブクローニングし、標準的な方法により増殖させることができる（Ｇｏｄｉｎｇ，Ｍｏｎｏｃｌｏｎａｌ Ａｎｔｉｂｏｄｉｅｓ：Ｐｒｉｎｃｉｐｌｅｓ ａｎｄ Ｐｒａｃｔｉｃｅ，５９－１０３頁（Ａｃａｄｅｍｉｃ Ｐｒｅｓｓ，１９８６））。この目的に対して好適な培地は、例えば、Ｄ－ＭＥＭ又はＲＰＭＩ－１６４０培地を包含する。また、該ハイブリドーマ細胞は、動物において腹水症腫瘍としてインビボで増殖させることができる。

サブクローンにより分泌されたモノクローナル抗体は、例えばプロテインＡ－セファロース、ヒドロキシアパタイトクロマトグラフィー、ゲル電気泳動、透析、又はアフィニティークロマトグラフィーのような常套的な免疫グロブリン精製法により、培地、腹水、又は血清から好適に分離される。

【００５１】

モノクローナル抗体をコードするＤＮＡは、常法を用いて（例えば、モノクローナル抗体の重鎖および軽鎖をコードしている遺伝子に特異的に結合できるオリゴヌクレオチドプローブを用いることにより）即座に分離され配列決定される。ハイブリドーマ細胞は、このようなＤＮＡの好ましい供給源となる。ひとたび分離されたならば、ＤＮＡを発現ベクター中に入れ、ついでこれを、この状況以外では免疫グロブリンタンパク質を産生しない大

腸菌細胞、サルＣＯＳ細胞、チャイニーズハムスター卵巣（ＣＨＯ）細胞、又は骨髄腫細胞のような宿主細胞中に形質移入し、組換え宿主細胞におけるモノクローナル抗体の合成を獲得することができる。ＤＮＡはまた、例えば、相同的マウス配列の代わりにヒト重鎖及び軽鎖定常部のコード化配列を置換することによって、Ｍｏｒｒｉｓｏｎら，Ｐｒｏｃ．Ｎａｔ．Ａｃａｄ．Ｓｃｉ．ＵＳＡ，８１：６８５１（１９８４））、又は免疫グロブリンコード化配列に非免疫グロブリンポリペプチドのコード化配列の全て又は一部を共有結合させることによって修飾することができる。そのように、「キメラ」又は「ハイブリッド抗体」は、ここでの抗－ｔｒｋモノクローナル抗体の結合特異性を有するように調製される。

【００５２】

典型的には、このような非免疫グロブリンポリペプチドは、抗体の定常部を置換、又は本発明の抗体の１個の抗原結合部位の定常部を置換し、ｔｒｋレセプターに対する特異性を有する１個の抗原結合部位、及び異なる抗原に対する特異性を有するもう一つの抗原結合部位を含むキメラ二価抗体を作り出す。

キメラ又はハイブリッド抗体は、また、架橋剤に関するものを含む、既知の合成タンパク質化学の方法を用いてインビトロで調製され得る。例えば、免疫毒素は、ジスルフィド交換反応を用いて、又はチオエーテル結合の形成によって構築され得る。この目的に関して適した試薬の例としては、イミノチオレート及びメチル－４－メルカプトブチリイミデートが含まれる。

抗体の組換え生産は以下に更に詳細に説明する。

【００５３】

（ｉｉｉ）ヒト化体

一般的に、ヒト化抗体には非ヒトを源にする一又は複数のアミノ酸残基が導入される。これら非ヒトアミノ酸残基は、しばしば、典型的には「移入」可変ドメインから得られる「移入」残基と称される。ヒト化は基本的にウィンター（ｗｉｎｔｅｒ）及び共同研究者［Ｊｏｎｅｓ等，Ｎａｔｕｒｅ，３２１：５２２－５２５（１９８６）；Ｒｉｅｃｈｍａｎｎ等，Ｎａｔｕｒｅ，３３２：３２３－３２７（１９８８）；Ｖｅｒｈｏｅｙｅｎ等，Ｓｃｉｅｎｃｅ，２３９：１５３４－１５３６（１９８８）］の方法に従って、齧歯類ＣＤＲ又はＣＤＲ配列をヒト抗体の対応する配列に置換することにより実施される。

よって、このような「ヒト化」抗体は、無傷のヒト可変ドメインより実質的に少ない分が非ヒト種由来の対応する配列で置換されたキメラ抗体（Ｃａｂｉｌｌｙ，上掲）である。実際には、ヒト化抗体は典型的にはある程度のＣＤＲ残基及び場合によってはいくらかのＦＲ残基が齧歯類抗体の類似する部位からの残基によって置換されるヒト抗体である。

【００５４】

抗体が、抗原及び他の好ましい生物学的特性に対する高い親和性の保持をともなってヒト化されることは重要である。この目的を達成するために、好ましい方法によって、ヒト化抗体は、親及びヒト化配列の三次元モデルを用いて、親配列及び概念上のヒト化産物の分析の工程によって調製される。三次元免疫グロブリンモデルは、広く入手可能であり、当該分野に熟練した者にとっては良く知られている。可能選択された候補免疫グロブリン配列の可能性のある三次元立体配置構造を例示して表示するコンピュータープログラムが入手可能である。これら表示の検討によって、候補免疫グロブリン配列の機能化における残基の可能性ある役割の分析が可能になる、すなわち、候補免疫グロブリンのその抗原へ結合する能力に影響を及ぼす残基の分析である。この方法では、標的抗原に対して増大した親和性のような所望される抗体特性が達成されるように、ＦＲ残基がコンセンサス及び移入配列から選択されて組み合わされることが可能である。一般的に、ＣＤＲ残基は、抗原結合に影響を与えることに、直接にそしてかなり十分に関与している。さらなる詳細については、１９９１年６月１４日に出願された米国出願第０７／７１５，２７２号と部分的に連続している１９９２年８月２１日に出願された米国出願特許第０７／９３４，３７３号を参照せよ。

【００５５】
（ｉｖ）ヒト抗体
ヒトモノクローナル抗体は、ハイブリドーマ法によって作製することができる。ヒトモノクローナル抗体の生産のためのヒトミエローマ及びマウス－ヒトヘテロミエローマ細胞株は，例えば，Ｋｏｚｂｏｒ，Ｊ．Ｉｍｍｕｎｏｌ．１３３，３００１（１９８４），及びＢｒｏｄｅｕｒ，ら．，Ｍｏｎｏｃｌｏｎａｌ Ａｎｔｉｂｏｄｙ Ｐｒｏｄｕｃｔｉｏｎ Ｔｅｃｈｎｉｑｕｅｓ ａｎｄ Ａｐｐｌｉｃａｔｉｏｎｓ，ｐｐ．５１－６３（Ｍａｒｃｅｌ Ｄｅｋｋｅｒ，Ｉｎｃ．，Ｎｅｗ Ｙｏｒｋ，１９８７）によって記載されている。 免疫化することで、内因性免疫グロブリンの生産なしに、ヒト抗体のレパートリーを生産することが可能なトランスジェニック動物（例えばマウス）を生産することが現在は可能である。例えば、キメラ及び生殖細胞系変異体マウスでの抗体重鎖結合領域（J_H）遺伝子のホモ接合体欠失は、内因性抗体の生産の完全な阻害をもたらすことが記載されている。そのような生殖細胞系変異体マウスでのヒト生殖細胞系免疫グロブリン遺伝子配列の転移は、抗原の挑戦によってヒト抗体の生産を引き起こす。例えば、Ｊａｋｏｂｏｖｉｔｓら．，Ｐｒｏｃ．Ｎａｔｌ．Ａｃａｄ．Ｓｃｉ．ＵＳＡ ９０，２５５１－２５５（１９９３）；Ｊａｋｏｂｏｖｉｔｓら．，Ｎａｔｕｒｅ ３６２，２５５－２５８（１９９３）を参照せよ。
【００５６】
Ｍｅｎｄｅｚら．（Ｎａｔｕｒｅ Ｇｅｎｅｔｉｃｓ １５：１４６－１５６［１９９７］）は、さらに技術を改良し、抗原の挑戦を受けた際に、高親和性の完全なヒト抗体を生成する「ＸｅｎｏｍｏｕｓｅＩＩ」と命名されたトランスジェニックマウスの系統を作製した。これは、上に記載したような内因性のJ_Hセグメントへの欠損をともなうマウスへのメガベースヒト重鎖及び軽鎖遺伝子座の生殖細胞系組み込みによって達成された。ＸｅｎｏｍｏｕｓｅＩＩは、およそ６６V_H遺伝子、完全なD_H及びJ_H領域及び３つの異なる定常領域（μ，δ及びχ）を含む１，０２０ｋｂのヒト重鎖遺伝子座を有し、そしてまた、３２Ｖκ遺伝子、Ｊκセグメント及びＣκ遺伝子を含む８００ｋｂのヒトκ遺伝子座を有する。これらのマウスで生産される抗体は、遺伝子再構成、構築及びレパートリーを含む全ての点でヒトにおいて見られるものと極めて類似している。ヒト抗体は、マウス遺伝子座での遺伝子再構成を防ぐ内因性のＪＨセグメントの欠損のために、内因性抗体より優先的に多く発現される。
【００５７】
あるいは、ファージディスプレイ技術（ＭａＣａｆｆｅｒｔｙら．，Ｎａｔｕｒｅ ３４８，５５２－５５３［１９９０］）を、免疫化されていない供与体の免疫グロブリン可変ドメイン遺伝子レパートリーからヒト抗体及び抗体断片をインビトロで生産することに用いることができる。この技術によると、抗体Ｖドメインは、Ｍ１３又はｆｄのような糸状バクテリオファージの主要又は副コートタンパク質遺伝子のいずれかへインフレームでクローンされ、ファージ粒子の表面上に機能性抗体断片として表示される。糸状粒子がファージゲノムの一本鎖ＤＮＡコピーを含むことから、抗体の機能的特性に基づいた選択も、これらの特性を示す抗体をコードする遺伝子の選択をする結果となる。従って、ファージは、Ｂ細胞の幾つかの特性を模倣する。ファージディスプレイは、種々のフォーマットでおこなうことができる；それらの概説として、例えば、Ｊｏｈｎｓｏｎ，Ｋｅｖｉｎ Ｓ．及びＣｈｉｓｗｅｌｌ，Ｄａｖｉｄ Ｊ．，Ｃｕｒｒｅｎｔ Ｏｐｉｎｉｏｎ ｉｎ Ｓｔｒｕｃｔｕｒａｌ Ｂｉｏｌｏｇｙ ３，５６４－５７１（１９９３）を参照せよ。Ｖ遺伝子セグメントの幾つかのソースをファージディスプレイのために用いることができる。Ｃｌａｃｋｓｏｎら．，Ｎａｔｕｒｅ ３５２，６２４－６２８（１９９１）は、免疫化したマウスの脾臓から取り出したＶ遺伝子の小さなランダムコンビナトリアルライブラリから、多様な抗－オキサゾロン抗体を単離した。免疫化していないヒト供与体のＶ遺伝子のレパートリーを構築することができ、多様な抗原（自己抗原を含む）に対する抗体を、基本的にＭａｒｋｓら．，Ｊ．Ｍｏｌ．Ｂｉｏｌ．２２２，５８１－５９７（１９９１）、又はＧｒｉｆｆｉｔｈら．，ＥＭＢＯ Ｊ．１２，７２５－７３４（１

９９３）によって記載されている技術に従って単離することができる。天然免疫応答では、抗体遺伝子は高速度で変異を蓄積する（体細胞過剰変異）。導入された幾つかの変化は、より高い親和性を与え、そして高親和性表面免疫グロブリンを表示するＢ細胞は、後の抗原の挑戦の間に、優先的に複製されて分化する。この天然プロセスは、「チェーン・シャッフリング」として知られている技術を用いて模倣することができる（Ｍａｒｋｓら．，Ｂｉｏ／Ｔｅｃｈｎｏｌ．１０，７７９－７８３［１９９２］）。この方法では、ファージディスプレイによって得られた「一次」ヒト抗体の親和性を、重鎖及び軽鎖Ｖ領域遺伝子を、免疫化していない供与体から得られたＶドメイン遺伝子の天然発生変異体（レパートリー）のレパートリーで経時的に置き換えることによって改良することができる。この技術は、ｎＭの程度での親和性で、抗体及び抗体断片の生産を可能にする。かなり大きなファージ抗体レパートリーを作製するための戦略（「全てのライブラリのもと」としても知られている）は、Ｗａｔｅｒｈｏｕｓｅら．，Ｎｕｃｌ．Ａｃｉｄｓ Ｒｅｓ．２１，２２６５－２２６６（１９９３）によって記載され、そのような大きなファージライブラリからの直接の高親和性ヒト抗体を単離は、印刷中のＧｒｉｆｆｉｔｈら．，ＥＭＢＯ Ｊ．（１９９４）によって報告されている。ジーンシャッフリングは、また、げっ歯動物の抗体からヒト抗体を誘導するために用いられ、そのヒト抗体は、初発のげっ歯抗体に対して類似の親和性及び特異性を有する。「エピトープ・インプリンティング」とも呼ばれるこの方法によると、ファージディスプレイ技術によって得られたげっ歯抗体の軽鎖Ｖ遺伝子ドメイン遺伝子は、ヒトＶドメイン遺伝子のレパートリーで置換されて、げっ歯－ヒトキメラを作成する。抗原での選択は、機能性抗原結合部位を使うことができるヒト可変部の単離を引き起こす結果となる、すなわちエピトープは、パートナーの選択を支配（インプリント）する。残りのげっ歯Ｖドメインを置換するために工程が繰り返されると、ヒト抗体が得られる（１９９３年４月１日に発行のＰＣＴ特許出願国際公開９３／０６２１３を参照せよ）。ＣＤＲ移植によるげっ歯抗体の伝統的なヒト化とは違って、この技術によって、げっ歯起源のフレームワーク又はＣＤＲ残基を有しない完全なるヒト抗体を提供される。

【００５８】

（ｖ）二重特性抗体

二重特異性抗体は、少なくとも２つの異なる抗原に対して結合特異性を有するモノクローナル抗体、好ましくはヒトもしくはヒト化抗体である。本発明の場合においては、結合特異性の一方はアゴニスト抗体を提供するためのｔｒｋＣレセプターに対してであり、他方は任意の他の抗原に対してであり、好ましくは、その他のレセプター又はレセプターサブユニットに対してである。例えば、ｔｒｋＣレセプター及びニューロトロフィン、又はｔｒｋＣレセプター及びその他のｔｒｋレセプターと特異的に結合する二重特異性抗体は、本発明の範囲内にある。

二重特異性抗体を作成する方法は当該技術分野において周知である。伝統的には、二重特異性抗体の組換え生産は、二つの重鎖が異なる特異性を持つ二つの免疫グロブリン重鎖／軽鎖対の同時発現に基づく（Ｍｉｌｓｔｅｉｎ及びＣｕｅｌｌｏ，Ｎａｔｕｒｅ，３０５：５３７－５３９（１９８３））。免疫グロブリンの重鎖と軽鎖を無作為に取り揃えるため、これらハイブリドーマ（クアドローマ）は１０種の異なる抗体分子の潜在的混合物を生成し、その内の一種のみが正しい二重特異性構造を有する。正しい分子の精製は、アフィニティークロマトグラフィー工程によって通常達成される。同様の手順が１９９３年５月１３日公開の国際公開９３／０８８２９、及びＴｒａｕｎｅｃｋｅｒら，ＥＭＢＯ Ｊ．，１０：３６５５－３６５９（１９９１）に開示されている。

【００５９】

異なるそしてより好ましい方法によって、所望の結合特異性（抗体－抗原結合部位）を有する抗体可変ドメインを、免疫グロブリン定常ドメイン配列へ融合することができる。この融合は、好ましくは、少なくともヒンジ部、ＣＨ２及びＣＨ３領域の一部を含む免疫グロブリン重鎖定常ドメインとのものである。少なくとも一つの融合には、軽鎖結合に必要な部位を含む第一の重鎖定常領域（ＣＨ１）が存在することが望ましい。免疫グロブリン

重鎖融合体をコードするＤＮＡ、そして望むのであれば免疫グロブリン軽鎖を、別々の発現ベクターに挿入し、適当な宿主微生物へ同時形質移入する。これにより、組立に使用される三つのポリペプチド鎖の等しくない比率が最適な収率をもたらす態様において、三つのポリペプチドフラグメントの相互の割合の調節に大きな融通性が与えられる。しかし、少なくとも二つのポリペプチド鎖の等しい比率での発現が高収率をもたらすとき、又はその比率が特に重要性を持たないときは、２または３個全てのポリペプチド鎖のためのコード化配列を一つの発現ベクターに挿入することが可能である。このアプローチ法の好適な実施態様では、二重特異性抗体は、第一の結合特異性を有する一方のアームのハイブリッド免疫グロブリン重鎖と他方のアームのハイブリッド免疫グロブリン重鎖－軽鎖対（第二の結合特異性を提供する）ことからなる。二重特異性分子の半分にしか免疫グロブリン軽鎖がないと容易な分離法が提供されるため、この非対称的構造は、所望の二重特異性化合物を不要な免疫グロブリン鎖の組み合わせから分離することを容易にすることが分かった。このアプローチ法は、１９９４年３月３日に公開の国際公開９４／０４６９０に開示されている。

二重特異性抗体を産生する更なる詳細については、例えばＳｕｒｅｓｈら，Ｍｅｔｈｏｄｓ ｉｎ Ｅｎｚｙｍｏｌｏｇｙ，１２１：２１０（１９８６）を参照されたい。

【００６０】

（ｖｉ）ヘテロコンジュゲート抗体

ヘテロコンジュゲート抗体もまた本発明の範囲に入る。ヘテロコンジュゲート抗体は、２つの共有結合した抗体からなる。このような抗体は、例えば、免疫系細胞を不要な細胞に対してターゲティングさせるため［米国特許第４，６７６，９８０号］及びＨＩＶ感染の治療のために［国際公開 ９１／００３６０；国際公開 ９２／２００３７３；ＥＰ ０３０８９］提案されている。ヘテロコンジュゲート抗体は、任意の簡便な架橋方法を用いて作製してもよい。適した架橋剤は当該分野で良く知られており、米国特許第４，６７６，９８０号に、多くの架橋技術とともに開示されている。

【００６１】

（ｖｉｉ）抗体断片

ある実施態様では、抗－ｔｒｋＣ抗体（マウス、ヒト及びヒト化抗体、及び抗体変異体を含む）は抗体断片である。抗体断片を生産するために様々な技術が開発されている。伝統的には、これらの断片は、無傷の抗体のタンパク分解性消化を介して誘導される（例えば、Ｍｏｒｉｍｏｔｏら，Ｊｏｕｒｎａｌ ｏｆ Ｂｉｏｃｈｅｍｉｃａｌ ａｎｄ Ｂｉｏｐｈｙｓｉｃａｌ Ｍｅｔｈｏｄｓ ２４：１０７－１１７（１９９２）及びＢｒｅｎｎａｎら，Ｓｃｉｅｎｃｅ，２２９：８１（１９８５）を参照されたい）。しかし、これらの断片は現在は組換え宿主細胞により直接生産することができる。例えば、抗体断片は上において検討した抗体ファージライブラリから分離することができる。別法として、Ｆａｂ’－ＳＨ断片は大腸菌から直接回収することができ、化学的に結合して$F(ab')_2$断片を形成することができる（Ｃａｒｔｅｒら，Ｂｉｏ／Ｔｅｃｈｎｏｌｏｇｙ １０：１６３－１６７（１９９２））。以下の実施例に記載されているような他の実施態様では、$F(ab')_2$分子の組立を促進するロイシンジッパーＧＣＮ４を使用して$F(ab')_2$が形成される。他のアプローチ法では、$F(ab')_2$断片を組換え宿主細胞培養から直接分離することができる。抗体断片の生産のための他の方法は当業者には明らかであろう。

【００６２】

（ｖｉｉｉ）抗体のアミノ酸配列変異体

抗－ｔｒｋＣ抗体のアミノ酸配列変異体は、適当なヌクレオチド変化を抗－ｔｒｋＣ抗体ＤＮＡに導入することにより、又はペプチド合成により調製される。そのような変異体は、例えば、ここの例の抗－ｔｒｋＣ抗体のアミノ酸配列内の残基の欠失、及び／又は挿入及び／又は置換を含む。欠失、挿入、及び置換の任意の組み合わせは、最終構造物に達するまでなされるが、その最終構造物は所望の特徴を有する。また、アミノ酸変化は、グリコシル化部位の数の変化などの変異抗－ｔｒｋＣ抗体の翻訳後過程を変更しうる。

突然変異居誘発に適した抗－ｔｒｋＣ抗体の或る残基又は領域の同定のために有用な方法は、Ｃｕｎｎｉｎｇｈａｍ及びＷｅｌｌｓ（１９８９），Ｓｃｉｅｎｃｅ ２４４：１０８１－１０８５（１９８９）に記載されているように「アラニンスキャンニング突然変異誘発」と呼ばれる。ここで、標的残基の残基又は基が同定され（例えば、ａｒｇ，ａｓｐ，ｈｉｓ，ｌｙｓ，及びｇｌｕ等の荷電残基）、中性又は負荷電アミノ酸（最も好ましくはアラニン又はポリペプチドアニリン）に置換され、アミノ酸とｔｒｋＣ抗原との相互作用に影響を及ぼす。次いで置換に対する機能的感受性を示すこれらのアミノ酸の位置は、置換部位において又はそれに対して更に又は他の置換を導入することにより精密にされる。従って、アミノ酸配列変異を導入する部位は予め決定されるが、変異自体の性質は予め決める必要はない。例えば、与えられた部位における性能を分析するために、ａｌａスキャンニング又はランダム突然変異誘発を標的コドン又は領域で実施し、発現された抗体変異体を所望の活性についてスクリーニングする。

【００６３】

アミノ酸配列挿入は、１残基から１００以上の残基を含むポリペプチドの長さの範囲のアミノ－及び／又はカルボキシル末端融合物、並びに一又は複数のアミノ酸残基の配列内挿入物を含む。末端挿入物の例は、Ｎ－末端メチオニル残基を持つ抗体又はエピトープタグに融合した抗体を含む。抗体分子の他の挿入変異体は、抗体の血清半減期を向上させる酵素又はポリペプチド又はＰＥＧの抗体のＮ－又はＣ－末端への融合物を含む（下記を参照せよ）。

他の型の変異体はアミノ酸置換変異体である。これらの変異体は、抗－ｔｒｋＣ抗体分子において少なくとも一つのアミノ酸残基が除去され、その位置に異なる残基が挿入されている。置換突然変異について最も興味深い部位は高頻度可変領域を含むが、ＦＲ変更も考慮される。保存的置換は、「好ましい置換」と題して表１に示す。これらの置換が生物学的活性の変化をもたらす場合、表Ａに「例示的置換」と名前を付けた又はアミノ酸の分類を参照して以下に更に記載するような、より実質的な変化を導入し、生成物をスクリーニングしてよい。

【００６４】

表１

元の残基	例示的置換	好ましい置換
Ala(A)	val; Leu; ile	val
Arg(R)	lys; gln; asn	lys
Asn(N)	gln; his; lys; arg	gln
Asp(D)	glu ; asn	glu
Cys(C)	ser ; ala	ser
Gln(Q)	asn; glu	asn
Glu(E)	asp; gln	asp
Gly(G)	ala	ala
His(H)	asn; gln; lys; arg	arg
Ile(I)	leu; val; met; ala; phe;ノルロイシン	leu
Leu(L)	ノルロイシン; ile; val; met; ala; phe	ile
Lys(K)	arg; gln; asn	arg
Met(M)	leu; phe; ile	leu
Phe(F)	leu; val; ile; ala; tyr	tyr
Pro(P)	ala	ala
Ser(S)	thr	thr
Thr(T)	ser	ser
Trp(W)	tyr; phe	tyr
Tyr(Y)	trp; phe; thr; ser	phe
Val(V)	ile; leu; met; phe; ala; ノルロイシン	leu

【００６５】
抗体の機能及び免疫学的同一性の置換的修飾は、（ａ）置換領域のポリペプチド骨格の構造、例えばシート又は螺旋配置、（ｂ）標的部位の電荷又は疎水性、又は（ｃ）側鎖の嵩を維持しながら、それらの効果において実質的に異なる置換基を選択することにより達成される。天然発生残基は共通の側鎖特性に基づいてグループに分けることができる：
（１）疎水性：ノルロイシン，ｍｅｔ，ａｌａ，ｖａｌ，ｌｅｕ，ｉｌｅ；
（２）中性の親水性：ｃｙｓ，ｓｅｒ，ｔｈｒ；
（３）酸性：ａｓｐ，ｇｌｕ；
（４）塩基性：ａｓｎ，ｇｌｎ，ｈｉｓ，ｌｙｓ，ａｒｇ；
（５）鎖配向に影響する残基：ｇｌｙ，ｐｒｏ；及び
（６）芳香族：ｔｒｐ，ｔｙｒ，ｐｈｅ。
【００６６】
非保存的置換は、これらの分類の一つのメンバーを他の分類に交換することを必要とするであろう。
ヒト化又は変異抗－ｔｒｋＣ抗体の適切な高次構造を維持することに関与していない任意のシステイン残基も、一般的にはセリンで置換されて、分子の酸化的安定性を向上させ、異常な架橋を防ぎ得る。逆に、システイン結合が抗体へ付与されて、その安定性が向上し得る（特に、抗体がＦｖ断片のような抗体断片である）。
【００６７】
置換変異の特に好ましい型には、親抗体（例えばヒト化又はヒト抗体）の一つ又は複数の

高頻度可変領域の残基を置換することが含まれる。一般的には、さらなる発展のために選択された結果として生じた変異は、それらが生成された親抗体に関連して生物学的特性が改良されている。そのような置換変異を生成するための簡便な方法は、ファージディスプレイを用いたアフィニティ成熟である。要約すると、幾つかの高頻度可変領域部位（例えば６－７部位）が変異されて、各部位で全ての可能なアミノ酸置換が生成される。従って、生成された抗体変異体は、各粒子内にパッケージされたＭ１３のｇｅｎｅ ＩＩＩ産物との融合物である糸状ファージ粒子から一価の様式で表示される。ファージディスプレイ変異体を、次いで、ここに開示されているように、その生物学的活性（例えば結合親和性）についてスクリーニングした。修飾のための候補高頻度可変領域部位を同定するために、アラニンスキャンニング突然変異誘発を、抗原結合に顕著に貢献している高頻度可変領域残基を同定するためにおこなった。あるいは、又はさらには、抗体とヒトｔｒｋＣの間の接触点を同定するために、抗原－抗体複合体の結晶構造を分析することは有益である。そのような接触残基及び隣接残基は、ここで詳しく記載された技術による置換の候補である。そのような変異体が一度生成されると、変異体のパネルは、ここで記載されているようにスクリーニングに供され、一つ又は複数の関連したアッセイで優れた特性を有する抗体は、さらなる展開のために選択される。

【００６８】

（ｉｘ）抗体のグリコシル化変異体

抗体は、その定常領域の保存的位置でグリコシル化されている（Ｊｅｆｆｅｒｉｓ及びＬｕｎｄ，Ｃｈｅｍ．Ｉｍｍｕｎｏｌ．６５：１１１－１２８［１９９７］；Ｗｒｉｇｈｔ及びＭｏｒｒｉｓｏｎ，ＴｉｂＴＥＣＨ １５：２６－３２［１９９７］）。免疫グロブロリンのオリゴ糖側鎖は、タンパク質の機能（Ｂｏｙｄら．，Ｍｏｌ．Ｉｍｍｕｎｏｌ．３２：１３１１－１３１８［１９９６］；Ｗｉｔｔｗｅ及びＨｏｗａｒｄ，Ｂｉｏｃｈｅｍ．２９：４１７５－４１８０［１９９０］）、及び糖タンパク質の高次構造と提示されている三次元表面に影響を与える糖タンパク質の部分間の分子内相互作用に影響を与える（Ｈｅｆｆｅｒｉｓ及びＬｕｎｄ，上掲；Ｗｙｓｓ及びＷａｇｎｅｒ，Ｃｕｒｒｅｎｔ Ｏｐｉｎ．Ｂｉｏｔｅｃｈ．７：４０９－４１６［１９９６］）。オリゴ糖は、また、特異的な認識構造に基づいて、所定の糖タンパク質をある分子へ標的とすることを担い得る。例えば、ガラクトシル化されたＩｇＧでは、オリゴ糖部分がＣＨ－２間スペースから飛び出し、末端Ｎ－アセチルグルコサミン残基は、マンノース結合タンパク質との結合が可能となる（Ｍａｌｈｏｔｒａら．，Ｎａｔｕｒｅ Ｍｅｄ．１：２３７－２４３［１９９５］）。グリコペプチダーゼによるＣＡＭＰＡＴＨ－１Ｈ（ヒトリンパ球のＣＤｗ５２抗原を認識する、組み換えヒト化マウスモノクローナルＩｇＧ１抗体）からのオリゴ糖の除去によって、チャイニーズ・ハムスター卵巣（ＣＨＯ）細胞は補体媒介溶解（ＣＭＣＬ）の完全縮小を生じ（Ｂｏｙｄら．，Ｍｏｌ．Ｉｍｍｕｎｏｌ．３２：１３１１－１３１８（１９９６））、一方では、ノイラミニダーゼを用いてシアル酸残基の選択的除去によってはＤＭＣＬの損失を生じない。特に、ＧｌｃＮＡｃを二等分する触媒形態のグリコシルトランスフェラーゼである、β（１，４）－Ｎ－アセチルグリコサミントランスフェラーゼＩＩＩ（ＧｎＩＩＩ）のテトラサイクリン制御発現のあるＣＨＯ細胞では、ＡＤＣＣ活性が向上したと報告された（Ｕｍａｎａら．，Ｍａｔｕｒｅ Ｂｉｏｔｅｃｈ．１７：１７６－１８０［１９９９］）。

【００６９】

抗体のグリコシル化変異体は、抗体のグリコシル化パターンが改変された変異体である。改変とは、抗体に見出された一つ又は複数の炭水化物部分を除去し、その抗体へ一つ又は複数の炭水化物部分を加え、グリコシル化の組成（グリコシル化パターン）、グリコシル化の程度等を変化させることである。例えば、グリコシル化変異体は、抗体をコードしている核酸配列の一つ又は複数のグリコシル化部位を取り除き、変化させ及び／又は加えることで調製し得る。

抗体のグリコシル化とは、典型的にはＮ－結合又はＯ－結合のいずれかである。Ｎ－結合とは、アスパラギン残基の側鎖への炭水化物部分の付与を指す。トリペプチドは、Ｘがプ

ロリンを除く任意のアミノ酸である、アスパラギン－Ｘ－セリン及びアスパラギン－Ｘ－スレオニンの配列であり、アスパラギン側鎖への炭水化物部分が酵素的に付与される認識部位である。従って、ポリペプチドのこれらトリペプチド配列のいずれかの存在によって、潜在的なグリコシル化部位を作り出される。Ｏ－結合グリコシル化とは、５－ヒドロキシルプロリン又は５－ヒドロキシルリジンも用いられるが、殆どの場合にはセリン又はスレオニンへＮ－アセチルガラクトサミン、ガラクトース、又はキシロースのうちの一つの糖を付与することを指す。

【００７０】

抗体へのグリコシル化部位の付加は、アミノ酸配列を改変して、それが上記に記載のトリペプチド配列（Ｎ－結合グリコシル化部位について）の一つ又は複数を含むようにすることによって簡便に完遂できる。この改変は、また、最初の抗体の配列へ一つ又は複数のセリン又はスレオニン残基を付加、又は置換することによって生成される（Ｏ－結合グリコシル化部位について）。

抗－ｔｒｋＣ抗体のアミノ酸配列変異体をコードする核酸分子は、当該分野で知られている種々の方法によって調製される。これらの方法には、限定されないが、オリゴヌクレオチド媒介（又は部位特異的）突然変異誘発、ＰＣＲ突然変異誘発、及び抗－ｔｒｋＣ抗体の早期に調製した変異体又は非変異体形のカセット突然変異誘発による、天然源からの単離（天然発生アミノ酸配列変異体の場合）又は調製を含む。

【００７１】

抗体のグリコシル化（グリコシル化パターンを含む）は、また、内在するヌクレオチド配列を改変せずに改変し得る。グリコシル化は、抗体を発現するために用いる宿主細胞に大きく依存する。潜在的な治療学としての、組み換え糖タンパク質の発現に用いられる細胞の型、例えば抗体が、希に天然細胞であることから、抗体のグリコシル化パターンの顕著な変化を期待することができる（例えば、Ｈｅｓら．，Ｊ．Ｂｉｏｌ．Ｃｈｅｍ．２７２：９０６２－９０７０［１９９７］を参照せよ）。宿主細胞の選択に加えて、抗体の組み換え生産の間にグリコシル化へ影響する因子には、成長形態、培地、製剤、培養密度、酸素添加、ｐＨ、精製概要等が含まれる。オリゴ糖生産に関わる特定の酵素の導入又は過剰発現を含む、種々の方法が特定の宿主生物で達成されるグリコシル化パターンを改変するために提案されている（米国特許第５，０４７，３３５号；５，５１０，２６１号及び５，２７８，２９９号）。グリコシル化、又はある型のグリコシル化は、例えばエンドグリコシダーゼＨ（ＥｎｄｏＨ）を用いて、糖タンパク質から酵素的に除くことができる。さらには、組み換え宿主細胞は、例えばある型の多糖類のプロッセッシングを欠損させることで、遺伝的に操作することができる。これら及び類似の技術が、当該分野で良く知られている。

【００７２】

抗体のグリコシル化構造は炭水化物分析の簡便な技術によって容易に分析でき、それには、レクチンクロマトグラフィー、ＮＭＲ、質量分析、ＨＰＬＣ、ＧＰＣ、単糖類組成分析、逐次酵素消化、及び高ｐＨアニオン交換クロマトグラフィーを用いて電荷に基づいてオリゴ糖を単離するＨＰＡＥＣ－ＰＡＤが含まれる。分析的目的のためにオリゴ糖を遊離させる方法も知られており、限定されないが、酵素処理（ペプチド－Ｎ－グリコシダーゼ、Ｆ／エンド－β－ガラクトシダーゼを用いて広くおこなわれた）、主にＯ－結合構造を遊離させるために激しいアルカリ環境を利用しての除去、及び無水ヒドラジンを利用した化学的方法でＮ－及びＯ－結合オリゴ糖の双方を遊離させることが含まれる。

【００７３】

（ｘ）抗体の他の修飾

ここで開示されている抗－ｔｒｋＣ抗体は、免疫リポソームとして処方することもできる。抗体を含有するリポソームは、例えばＥｐｓｔｅｉｎら，Ｐｒｏｃ．Ｎａｔｌ．Ａｃａｄ．Ｓｃｉ．ＵＳＡ ８２：３６８８（１９８５）；Ｈｗａｎｇら，Ｐｒｏｃ．Ｎａｔｌ．Ａｃａｄ．Ｓｃｉ．ＵＳＡ ７７：４０３０（１９８０）；及び米国特許第４，４８５，０４５号及び同４，５４４，５４５号に記載されているように、当該分野に

おいて既知の方法により調製される。循環時間が増したリポソームは米国特許第５，０１３，５５６号に開示されている。
特に有用なリポソームは、ホスファチジルコリン、コレステロール及びＰＥＧ－誘導体化ホスファチジルエタノールアミン（ＰＥＧ－ＰＥ）を含有する脂質組成物を用いた逆相蒸発法により作製することができる。リポソームは孔径が定められたフィルターを通して押し出され、所望の直径を有するリポソームが得られる。本発明の抗体のＦａｂ’フラグメントは、ジスルフィド交換反応を介して、Ｍａｒｔｉｎら，Ｊ．Ｂｉｏｌ．Ｃｈｅｍ．２５７：２８６－２８８（１９８２）に記載されているようにしてリポソームにコンジュゲートすることができる。場合によっては、化学療法剤はリポソーム内に包含される。Ｇａｂｉｚｏｎら，Ｊ．Ｎａｔｉｏｎａｌ Ｃａｎｃｅｒ Ｉｎｓｔ．８１（１９）１４８４（１９８９）を参照されたい。

【００７４】

また、本発明の抗体は、プロドラッグ（例えばペプチジル化学療法剤、国際公開８１／０１１４５号を参照）を活性な抗癌剤に転化させるプロドラッグ活性化酵素に抗体をコンジュゲートさせることにより、ＡＤＥＰＴにおいて使用することができる。例えば国際公開８８／０７３７８及び米国特許第４９７５２７８号を参照されたい。
ＡＤＥＰＴに有用な免疫コンジュゲートの酵素成分には、より活性な細胞毒形態に転化するように、プロドラッグに作用し得る任意の酵素が含まれる。

【００７５】

限定するものではないが、この発明の方法に有用な酵素には、ホスファート含有プロドラッグを遊離の薬剤に転化するのに有用なアルカリ性ホスファターゼ；スルファート含有プロドラッグを遊離の薬剤に転化するのに有用なアリールスルファターゼ；非毒性５－フルオロシトシンを抗癌剤５－フルオロウラシルに転化するのに有用なシトシンデアミナーゼ；プロテアーゼ、例えばセラチアプロテアーゼ、サーモリシン、サブチリシン、カルボキシペプチダーゼ及びカテプシン（例えば、カテプシンＢ及びＬ）で、ペプチド含有プロドラッグを遊離の薬剤に転化するのに有用なもの；Ｄ－アミノ酸置換基を含有するプロドラッグの転化に有用なＤ－アラニルカルボキシペプチダーゼ；炭水化物切断酵素、例えばグリコシル化プロドラッグを遊離の薬剤に転化するのに有用なノイラミニダーゼ及びβガラクトシダーゼ；βラクタムで誘導体化された薬剤を遊離の薬剤に転化させるのに有用なβラクタマーゼ；及びペニシリンアミダーゼ、例えばそれぞれフェノキシアセチル又はフェニルアセチル基で、それらのアミン性窒素において誘導体化された薬剤を遊離の薬剤に転化するのに有用なペニシリンＶアミダーゼ又はペニシリンＧアミダーゼが含まれる。あるいは、「アブザイム」としてもまた公知の酵素活性を有する抗体を、遊離の活性薬剤に本発明のプロドラッグを転化させるために使用することもできる（例えば、Ｍａｓｓｅｙ，Ｎａｔｕｒｅ ３２８：４５７－４５８［１９８７］を参照）。抗体－アブザイムコンジュゲートは、ここで記載されているようにして、腫瘍細胞個体群にアブザイムを送達するために調製することができる。

【００７６】

この発明の酵素は、当該分野においてよく知られている技術、例えば上で検討したヘテロ二官能性架橋試薬を使用することにより、抗－ｔｒｋＣ抗体に共有的に結合させることができる。あるいは、本発明の抗体の少なくとも結合領域を本発明の酵素の少なくとも機能的に活性な部位に結合せしめてなる融合タンパク質を、当該技術においてよく知られている組換えＤＮＡ技術を使用して作成することができる（Ｎｅｕｂｅｒｇｅｒら，Ｎａｔｕｒｅ ３１２：６０４－６０８［１９８４］）。
本発明のある実施態様においては、無傷の抗体よりも抗体フラグメントを使用することが望ましい。この場合、その血清半減期を増大させるために抗体フラグメントを改変することが望ましい。これは、例えば、抗体フラグメントにサルベージレセプター結合エピトープを導入することにより（例えば、抗体フラグメント中の適当な領域の突然変異により、あるいはついで抗体フラグメントの何れかの末端又は中央に、例えばＤＮＡ又はペプチド合成により融合されるペプチドタグ内にエピトープを導入することにより）、達成できる

。１９９６年１０月１７日に発行の国際公開９６／３２４７８号を参照せよ。
【００７７】
サルベージレセプター結合エピトープは、一般的には、Ｆｃドメインの一又は二つのループからの一又は複数のアミノ酸残基が抗体断片の類似位置に移される領域を構成する。更により好ましくは、Ｆｃドメインの一又は二つのループの３又はそれ以上の残基が移される。なお更に好ましくは、エピトープはＦｃ領域（例えばＩｇＧの）のＣＨ２ドメインから取上げられ、抗体のＣＨ１、ＣＨ３、又はV_H領域、あるいは一以上のそのような領域に移される。別法として、エピトープをＦｃ領域のＣＨ２ドメインから取上げ、抗体断片のC_L領域又はV_L領域、又は両方に移す。
【００７８】
ヒト化又は変異抗－ｔｒｋＣ抗体（グリコシル化変異体を含む）の共有結合修飾も本発明の範囲内に含まれる。それらは、化学合成により、又は適用可能ならば抗体の酵素的もしくは化学的開裂により製造し得る。他のタイプの抗体の共有結合修飾は、抗体の標的とするアミノ酸残基を、選択した側鎖又はＮもしくはＣ末端残基と反応できる有機の誘導体化剤と反応させることにより、分子中に導入される。ポリペプチド共有結合修飾の例は、特にここに出典を明示して取り込む米国特許第５，５３４，６１５号に記載されている。抗体の共有結合修飾の好適なタイプは、米国特許第４，６４０，８３５号；同４，４９６，６８９号；同４，３０１，１４４号；同４，６７０，４１７号；同４，７９１，１９２号又は同４，１７９，３３７号に記載されたようにして、様々な非タンパク様ポリマー、例えばポリエチレングリコール、ポリプロピレングリコール、又はポリオキシアルキレンの１つに抗体を結合させることから構成される。
【００７９】
２．ベクター，宿主細胞及び組み換え方法
本発明は、また、非ヒトをコードする単離された核酸、例えばマウス及び本発明のヒト抗－ｔｒｋＣ抗体（非ヒト抗体のヒト化異形を含む）、核酸を含むベクター及び宿主細胞、そしてその抗体を生産するための組み換え技術を提供する。　抗体の組み換え生産に関しては、さらなるクローニング（ＤＮＡの増幅）又は発現のために、それをコードする核酸を単離して複製ベクター挿入することができ得る。その他の実施態様では、特に参考文献によってここで取り入れられている、例えば米国特許第５，２０４，２４４号に記載されている、相同組み換えによって抗体を生産させてもよい。モノクローナル抗体をコードするＤＮＡは、簡便な方法（例えば、抗体の重鎖及び軽鎖をコードする遺伝子と特異的に結合することが可能なオリゴヌクレオチドプローブを用いることによって）を用いて容易に単離及び配列決定できる。多くのベクターが入手可能である。限定されないが、ベクター成分には一般的に次の一つ又は複数ものが含まれる：１９９６年７月９日に発行の米国特許第５，５３４，６１５号に記載され、文献によって特にここで取り入れられている、単一の配列、複製開始点、一つ又は複数のマーカー遺伝子、エンハンサーエレメント、プロモーター、そして転写終結配列。
【００８０】
ここでのベクター中でのクローニング又はＤＮＡの発現に適した宿主細胞は、上記の原核生物、酵母、又は高等真核細胞である。この目的に対して好適な原核生物は、エシェリキア、例えば大腸菌、エンテロバクター、エルウイニア、クレブシエラ、プロテウス、サルモネラ、例えばサルモネラ・チフィムリウム、セラチア、例えばセラチア・マルセスセンス、及びシゲラ、並びにバシラス、例えばバシラス・サチリス及びバシラス・リチエニホルミス（例えば１９８９年４月１２日公開のＤＤ２６６７１０に開示されたバシラス・リチエニホルミス４１Ｐ）、シュードモナス、例えばシュードモナス・エールギノーサ、及びストレプトマイセスのような腸内細菌のごときグラム陰性とグラム陽性のような真性細菌を含む。１つの好ましい大腸菌クローニング宿主は、大腸菌２９４（ＡＴＣＣ３１，４４６）であるが、大腸菌Ｂ、大腸菌Ｘ１７７６（ＡＴＣＣ３１，５３７）、及び大腸菌Ｗ３１１０（ＡＴＣＣ２７，３２５）も適している。これらの例は、限定的でなく、例示的である。

【００８１】
原核生物に加えて、糸状菌や酵母のような真核性微生物が抗－ｔｒｋＣ抗体コードベクターの好適なクローニング又は発現宿主である。一般のパン酵母であるサッカロミセス・セレビシエは下等真核宿主微生物の中で最もよく利用される。しかし、多くの他の属、種、及び株も一般に利用でき、ここで有用であり、例えば、シゾサッカロミセス・ポンベ；例えばクルイベロミセス・ラクチス、クルイベロミセス・フラジリス（ＡＴＣＣ１２，４２４）、クルイベロミセス・ブルガリクス（ＡＴＣＣ１６，０４５）、クルイベロミセス・ビッケラミー（ＡＴＣＣ２４，１７８）、クルイベロミセス・ワルティー（ＡＴＣＣ５６，５００）、クルイベロミセス・ドロソフィラルム（ＡＴＣＣ３６，９０６）、クルイベロミセス・サーモトレランス及びクルイベロミセス・マルキシアナスのようなクルイベロミセス宿主；ヤロービア（ＥＰ４０２，２２６）；ピチア・パストリス（ＥＰ１８３，０７０）；カンジダ；トリコデルマ・リーシア（ＥＰ２４４，２３４）；ニューロスポラ・クラッサ；シュバニオマイセス・オクシデンタリスのようなシュバニオマイセス；及び例えばニューロスポラ、ペニシリウム、トリポクラジウムのような糸状菌、及びアスペルギルス・ニジュランス及びアスペルギルス・ニガーのようなアスペルギルス宿主である。
【００８２】
グリコシル化抗－ｔｒｋＣ抗体の発現に適した宿主細胞は、多細胞生物から由来のものである。非脊椎動物細胞の例には植物及び昆虫細胞が含まれる。多くのバキュロウイルス株及び変異体及びスポドプテラ・フルジペルダ（毛虫）、アエデス・エジプテイ（蚊）、アエデス・アルボピクタス（蚊）、ドロソフィラ・メラノガスター（ミバエ）、及びボンビクス・モリのような宿主由来の対応する許容される昆虫宿主細胞が同定されている。種々のトランスフェクション用のウィルス株が、公に入手でき、例えばオートグラファ・カルフォルニカ（Ａｕｔｏｇｒａｐｈａ Ｃａｌｉｆｏｒｎｉｃａ）ＮＰＶのＬ－１変異株、ボンビクス・モリ（Ｂｏｍｂｙｘ ｍｏｒｉ）ＮＰＶのＢｍ－５株が挙げられ、このようなウィルスは、本発明に係るウィルスとして、特に、スポドプテラ・フルギペルダ細胞のトランスフェクションのために使用してもよい。綿、トウモロコシ、ジャガイモ、大豆、ペチュニア、トマト及びタバコの植物細胞培養も宿主として使用できる。
【００８３】
しかし、最大の関心は脊椎動物細胞に向けられ、培養（組織培養）した脊椎動物細胞の増殖がルーチン作業となった。有用な哺乳動物宿主細胞株の例は、ＳＶ４０（ＣＯＳ－７，ＡＴＣＣ ＣＲＬ １６５１）で形質転換させたサル腎ＣＶ１細胞株；ヒト胚芽腎細胞株（２９３又は懸濁培養で生長するようにサブクローン化された２９３細胞，Ｇｒａｈａｍら，Ｊ．Ｇｅｎ Ｖｉｒｏｌ．，３６：５９ （１９７７））；ベビーハムスター腎細胞（ＢＨＫ，ＡＴＣＣ ＣＣＬ１０）；チヤイニーズハムスター卵巣細胞／－ＤＨＦＲ（ＣＨＯ，Ｕｒｌａｕｂら，Ｐｒｏｃ．Ｎａｔｌ．Ａｃａｄ．Ｓｃｉ．ＵＳＡ ７７：４２１６（１９８０））；マウスセルトリ細胞（ＴＭ４，Ｍａｔｈｅｒ，Ｂｉｏｌ．Ｒｅｐｒｏｄ．，２３：２４３－２５１ （１９８０））；サル腎細胞（ＣＶ１ ＡＴＣＣ ＣＣＬ７０）；アフリカミドリザル腎細胞（ＶＥＲＯ－７６，ＡＴＣＣ ＣＲＬ－１５８７）；ヒト頚管腫瘍細胞（Ｈｅｌａ，ＡＴＣＣ ＣＣＬ２）；イヌ腎細胞（ＭＤＣＫ，ＡＴＣＣ ＣＣＬ３４）；バッファローラット腎細胞（ＢＲＬ ３Ａ，ＡＴＣＣ ＣＲＬ １４４２）；ヒト肺細胞（Ｗ１３８，ＡＴＣＣ ＣＣＬ ７５）；ヒト肝細胞（Ｈｅｐ Ｇ２，ＨＢ ８０６５）；マウス乳房腫瘍細胞（ＭＭＴ ０６０５６２，ＡＴＣＣ ＣＣＬ５１）；ＴＲＩ細胞（Ｍａｔｈｅｒら，Ａｎｎａｌｓ Ｎ．Ｙ．Ａｃａｄ．Ｓｃｉ．，３８３：４４－６８ （１９８２））；ＭＲＣ５細胞；ＦＳ４細胞である。
宿主細胞は、抗－ｔｒｋＣ抗体生産のために上述の本発明の発現又はクローニングベクターで形質転換され、プロモーターを誘発し、形質転換体を選出し、又は所望の配列をコードする遺伝子を増幅するために適切に修正した通常の栄養培地で培養される。
【００８４】
この発明の抗－ｔｒｋＣ抗体を製造するのに用いる宿主細胞は、様々な培地中で培養し得る。ＨａｍのＦ１０（シグマ）、最小必須培地（（ＭＥＭ）（シグマ））、ＲＰＭＩ－１

６４０（シグマ）、及びダルベッコの改変イーグル培地（（ＤＭＥＭ）（シグマ）等の商業的に入手し得る培地が、該宿主細胞を培養するのに適している。また、例えば、Ｈａｍら．，Ｍｅｔｈｏｄｓ ｉｎ Ｅｎｚｙｍｏｌｏｇｙ，５８：４４（１９７９）；Ｂａｒｎｅｓら．，Ａｎａｌ．Ｂｉｏｃｈｅｍ．，１０２：２５５（１９８０）；米国特許第４，７６７，７０４号、第４，６５７，８６６号、第４，９２７，７６２号；第５，１２２，４６９号；又は第５，１２２，４６９号；国際公開９０／０３４３０または同８７／００１９５号；又は再発行米国特許第３０，９８５号に記載されたいずれの培地も、該宿主細胞用の培地として使用し得る。これらの培地のいずれも、必要に応じて、ホルモンおよび／または他の成長因子（インスリン、トランスフェリン、又は上皮増殖因子等の）、塩（塩化ナトリウム、カルシウム、マグネシウム、およびリン酸塩等の）、緩衝液（ＨＥＰＥＳ等の）、ヌクレオシド（アデノシンおよびチミジン等の）、抗生物質（ゲンタマイシン剤（商品名）等の）、微量元素（最終濃度がマイクロモルの範囲で通常存在する無機化合物）、およびグルコースまたは等価なエネルギー源を補い得る。いずれの他の必要な添加物も、当業者に知られているであろう適当な濃度で含めてもよい。温度、ｐＨ等の培養条件は、発現用に選択された宿主細胞について以前に用いられた条件であり、当業者には明らかであろう。

【００８５】

組換え技術を使用する場合、抗体は、細胞膜周辺腔において細胞内生産することができ、又は培地に直接分泌させることができる。抗体が細胞内生産されるときは、第一工程として、宿主細胞であれ溶解断片であれ、粒状細片を、例えば遠心分離又は限外濾過により除去する。Ｃａｒｔｅｒら，Ｂｉｏ／Ｔｅｃｈｎｏｌｏｇｙ １０：１６３－１６７（１９９２）には、大腸菌の細胞膜周辺腔に分泌される抗体を単離する手順が記載されている。簡単には、細胞ペーストを、酢酸ナトリウム（ｐＨ３．５）、ＥＤＴＡ、及びフェニルメチルスルホニルフロリド（ＰＭＳＦ）の存在下で、約３０分以上かけて溶かす。細胞屑は遠心分離により除去可能である。抗体が培地に分泌される場合、そのような発現系からの上清は、一般的に、商業的に入手可能なタンパク質濃縮フィルター、例えばアミコン又はミリポアペリコン限外濾過ユニットを使用して最初に濃縮される。ＰＭＳＦのようなプロテアーゼインヒビターを、タンパク分解を阻害するために先の工程の何れかで含めてもよく、また抗生物質を外来性汚染物の成長を防ぐために含めてもよい。

【００８６】

細胞から調製された抗体組成物は、例えばヒドロキシルアパタイトクロマトグラフィー、ゲル電気泳動法、透析、及びアフィニティークロマトグラフィーを使用して精製することができ、アフィニティークロマトグフィーが好適な精製法である。アフィニティーリガンドとしてのプロテインＡの適合性は、抗体中に存在する任意の免疫グロブリンＦｃドメインの種及びアイソタイプに依存する。プロテインＡはヒト１、２、又は４重鎖に基づく抗体を精製するために使用することができる（Ｌｉｎｄｍａｒｋら，Ｊ．Ｉｍｍｕｎｏｌ．Ｍｅｔｈ．６２：１－１３［１９８３］）。プロテインＧが全てのマウスのアイソタイプ及びヒト３に対して推奨される（Ｇｕｓｓら，ＥＭＢＯ Ｊ．５：１５６７－１５７５［１９８６］）。アフィニティーリガンドが結合するマトリックスは最も頻繁にはアガロースであるが、他のマトリックスも利用することができる。調整穴あきガラスやポリ（スチレンジビニル）ベンゼンのように機械的に安定なマトリックスを使用すれば、アガロースの場合よりも速い流速と短い処理時間が可能になる。抗体がC_H３ドメインを含む場合は、ベイカーボンド（Ｂａｋｅｒｂｏｎｄ）ＡＢＸ（商品名）樹脂（Ｊ．Ｔ．Ｂａｋｅｒ，ニュージャージー州フィリップスバーグ）が精製に有用である。イオン交換カラムによる分画、エタノール沈殿、逆相ＨＰＬＣ、シリカによるクロマトグラフィー、ヘパリンセファロース（ＳＥＰＨＡＲＯＳＥ（商品名））によるクロマトグラフィー、陰イオン又は陽イオン交換樹脂（ポリアスパラギン酸カラムなど）によるクロマトグラフィー、クロマトフォーカシング、ＳＤＳ－ＰＡＧＥ及び硫酸アンモニウム沈殿のような他のタンパク質精製技術も、回収しようとする抗体によっては利用できる。

【００８７】

前段階の精製工程に続いて、関心ある抗体と汚染物質を含有する混合物を、約２．５－４．５のｐＨで溶離バッファーを使用し、好ましくは低い塩分濃度（例えば、約０－０．２５Ｍの塩）で低ｐＨ疎水性相互作用クロマトグラフィーにかけてもよい。

【００８８】

３．アゴニスト抗－ｔｒｋＣ抗体の同定

アゴニスト抗体は、例えば、米国特許第５，７６６，８６３号及び５，８９１，６５０号に記載に記載のキナーゼレセプター活性化（ＫＩＲＡ）アッセイを用いて同定し得る。このＥＬＩＳＡ－型アッセイは、選択されたｒＰＴＫの潜在的アゴニスト又はアンタゴニストの同定及び特徴付けだけでなく、レセプタータンパク質チロシンキナーゼ（ｒＰＴＫ、例えばｔｒｋレセプター）のキナーゼドメインの自己リン酸化を測定することによるキナーゼ活性の質的又は量的測定にとって適している。アッセイの最初の段階には、レセプターが真核細胞の細胞膜に存在している、ｔｒｋＣレセプターの存在において、キナーゼレセプターのキナーゼドメインのリン酸化が含まれる。このレセプターは、レセプター、又はレセプター構成物をコードする内因性のレセプター又は核酸であってもよく、細胞へ形質転換してもよい。典型的には、最初の固相（例えば、最初のアッセイプレートのウェル）は、そのような細胞（通常は哺乳動物細胞株）の実質的に均一な集団でコーティングされて、細胞は固相へ付着される。しばしば、この細胞は接着性であり、よって最初の固相と自然に接着する。「レセプター構成物」が用いられた場合、通常は、キナーゼレセプターとフラッグポリペプチドの融合物が含まれる。このフラッグポリペプチドは、アッセイのＥＬＩＳＡの部分で、しばしば捕獲抗体である、捕獲剤によって認識される。候補アゴニストのような検体は、次に接着細胞を有するウェルへ添加され、チロシンキナーゼレセプター（例えばｔｒｋＣレセプター）が検体へ曝される（接触させられる）。このアッセイは、対象であるチロシンキナーゼレセプター（例えばｔｒｋＣ）に対するアゴニストリガンドの同定を可能にする。チロシンキナーゼレセプターのアンタゴニストを検出するために、このアッセイを用いることも可能である。アゴニストのレセプターへの結合を阻止するアンタゴニストリガンドの存在を検出するために、接着している細胞を最初に疑似アンタゴニストリガンド、次にアゴニストリガンドへ曝し、レセプター結合及び活性化の競争阻害を測定することができるようにする。また、このアッセイによって、アゴニストリガンドと結合し、それによって、そのｒＰＴＫと結合して活性化させる能力を減じて除去するアンタゴニストを同定することができる。そのようなアンタゴニストを検出するために、疑似アンタゴニスト及びｒＰＴＫに対するアゴニストをともにインキュベートし、次いで、リガンドのこの混合物へ接着細胞を曝す。検体へ曝した後、溶解バッファー（可溶化界面活性剤を有する）と緩やかな撹拌によって接着細胞を可溶化し、細胞可溶化液の濃縮又は浄化の必要なしに、アッセイのＥＬＩＳＡの部分へ直接に供することができる細胞可溶化液を遊離させる。

【００８９】

従って、調製された細胞可溶化液は、アッセイのＥＬＩＳＡの段階に供せる状態にある。ＥＬＩＳＡ段階での最初のステップのように、第二の固相（通常は、ＥＬＩＳＡマイクロプレートのウェル）を、チロシンキナーゼと特異的に、又はレセプター構成物の場合はフラッグポリペプチドと結合する捕獲剤（しばしば捕獲抗体）でコーティングされる。第二の固相のコーティングは、捕獲剤が第二の固相と接着するようにおこなわれる。捕獲剤は、一般的にはモノクローナル抗体であるが、ここでの実施例に記載されているように、ポリクローナル抗体も用いてもよい。得られた細胞可溶化液は、次いで接着剤へ曝す、又は接触させ、レセプター又はレセプター構成物が第二番目の固相へ接着（又は捕獲される）するようにする。次いで、洗浄段階を実行し、結合していない細胞可溶化液を取り除き、捕獲レセプター又はレセプター構成物を残した。接着又は捕獲レセプター又はレセプター構成物を、次いで、チロシンキナーゼレセプターのリン酸化されたチロシン残基を同定する抗－ホスホチロシン抗体へ曝す、又は接触させる。好ましい実施態様では、抗－ホスホチロシン抗体を、非放射呈色試薬の呈色変化を触媒する酵素と共役させる（直接又は間接）。従って、レセプターのリン酸化は、試薬のその後の呈色変化によって測定できる。こ

の酵素は、抗－ホスホチロシン抗体と直接に結合することができるか、又はコンジュゲート分子（例えばビオチン）は、抗－ホスホチロシン抗体と共役することができ、そしてこの酵素は、共役分子を介して抗－リン酸化チロシン抗体と後に結合することができる。最終的には、捕獲レセプター又はレセプター構成物と抗－リン酸化チロシン抗体の結合は、例えば呈色試薬の呈色変化によって測定できる。

【００９０】

最初の同定に続き、アゴニスト活性を、標的生物学的活性を試験することで知られている、バイオアッセイによってさらに確認し、精緻化することができる。例えば、ＮＴ－３の活性を模倣する抗－ｔｒｋＣモノクローナル抗体の能力は、実施例１に記載のようなＰＣ１２神経突起成長アッセイで試験することができ、実施例２に記載のシスプラチン－及びピリドキシン誘導神経障害の実験動物モデルのような既知の神経変性疾患の動物モデルで確かめることができる。

【００９１】

３．アゴニスト抗－ＴｒｋＣ抗体の治療的及び診断的用途

本発明の抗－ｔｒｋＣアゴニスト抗体は、細胞の変性又は機能障害に関与する疾患の症状の治療（予防を含む）に有用であると考えられている。特に、抗－ｔｒｋＣアゴニスト抗体は、種々の（慢性）神経変性障害及び急性神経細胞傷害の治療にとって有望な候補である。そのような神経変性疾患には、制限なく、末梢神経障害；運動ニューロン疾患、例えば筋萎縮性側索硬化症（ＡＬＳ、ルー・ゲーリック病）、顔面麻痺、及び脊髄筋萎縮症又は麻痺を含む種々の症状；及び他のヒト神経変性疾患、例えばアルツハイマー病、パーキンソン病、てんかん、多発性硬化症、ハンチントン舞踏病、ダウン症、神経性難聴、及びメニエール病、及び例えばトラウマ又は脊髄損傷による急性神経細胞損傷が含まれる。

【００９２】

本発明の抗－ｔｒｋＣ抗体は、末梢神経を冒す神経変性疾患で、殆どが運動、感覚、運動感覚、又は自律神経機能障害の一つ又は組み合わせである末梢神経障害の治療にとって特に適していると考えられている。例えば、末梢神経障害は、遺伝子的に獲得され、全身性疾患によりもたらされ、毒剤、例えば神経毒薬、例えば抗悪性腫瘍薬、又は産業性又は環境性汚染物質によって誘発される可能性がある。従って、末梢感覚神経障害は、末梢感覚ニューロンの機能の変性、減少又は不全によって特徴付けられ、それは、例えば糖尿病（糖尿病性ニューロパシー）、癌における抗悪性腫瘍薬治療（例えば、ビンクリスチン、シスプラチン、メトトレキサート、３’－アジド－３’－デオキシチミジンによる化学療法剤による治療）、アルコール依存症、エイズ，後天性免疫不全症候群（ＡＩＤＳ）、又は遺伝子的疾病素質の結果として起こり得る。遺伝的後天性抹消神経障害には、例えば、レフスム病、クラッベ病、異染性ロイコジストロフィー、ファブリー病、デジェリン・ソッタ病、無βリポタンパク質血症、及びシャルコー・マリー・ツース（ＣＭＴ）病（腓骨筋萎縮症又は遺伝性運動感覚性ニューロパチシー（ＨＭＳＮ）としても知られている）。

【００９３】

ｔｒｋＣレセプターの天然リガンドであるＮＴ－３の示された能力に基づいて、末梢血白血球の増殖を促進するために、本発明の抗－ｔｒｋＣアゴニスト抗体は、好中球減少症、種々の感染症、及び腫瘍の治療のための治療剤としても用いられる。ｔｒｋＣの発現がニューロンに限定されていないために、抗－ｔｒｋＣアゴニスト抗体は、神経細胞に制限することなく、一般的に細胞変性によって特徴付けられる疾患の予防又は治療での用途が見出せることが期待されている。

【００９４】

本発明の抗－ｔｒｋＣ抗体は、血管形成の誘導、又は血管形成の誘導が望まれる病理的症状／疾患の治療することにも用いるられ得る。そのような病理的症状には、例えば、不十分な脳循環によって生じる脳血管障害を含む潜在的な病理にも関わらず、心臓虚血が含まれる。血管形成は、潰瘍、鎌状赤血球病の糖尿病性合併症、及び外科手術後の創傷を含む創傷の治療においても所望され得る。

本発明の抗－ｔｒｋＣ抗体は、細胞変性、特に上記に掲載の神経変性疾患が関与する疾患

の診断において有用である。

【００９５】

診断用途については、抗体は、典型的には検出可能な成分で標識される。概して、次の分類に分類される多くの標識が入手可能である：

（ａ）放射性同位体、例えば、^{35}Ｓ、^{14}Ｃ、^{125}Ｉ、^{3}Ｈ及び^{131}Ｉ等。抗体は、例えばＣｕｒｒｅｎｔ Ｐｒｏｔｏｃｏｌｓ ｉｎ Ｉｍｍｕｎｏｌｏｇｙ，Ｖｏｌｕｍｅｓ １ ａｎｄ ２，Ｃｏｌｉｇｅｎ等，編，Ｗｉｌｅｙ－Ｉｎｔｅｒｓｃｉｅｎｃｅ，Ｎｅｗ Ｙｏｒｋ，Ｐｕｂｓ．（１９９１）に記載された技術を用いて放射性同位体で標識され、放射活性はシンチレーションカウンティングにより測定できる。

（ｂ）蛍光標識、例えば希土類キレート（ユーロピウムキレート）又はフルオレセイン及びその誘導体、ローダミン及びその誘導体、ダンシル、リサミン（Ｌｉｓｓａｍｉｎｅ）、フィコエリトリン及びテキサスレッド等が使用できる。蛍光標識は、例えば上掲のＣｕｒｒｅｎｔ Ｐｒｏｔｏｃｏｌｓ ｉｎ Ｉｍｍｕｎｏｌｏｇｙに開示された技術を用いて抗体に抱合させることができる。蛍光は、蛍光計を用いて定量できる。

（ｃ）種々の酵素－基質標識が利用でき、米国特許第４，２７５，１４９号は、それらの幾つかの概説を提供している。酵素は一般に、種々の技術を用いて測定可能な色素原基質の化学変換を触媒する。例えば、酵素は基質における色変化を触媒視、それは分光学的に測定可能である。あるいは、酵素は基質の蛍光又は化学発光を変化させることもある。蛍光変化を定量化する技術は上述した。化学発光基質は化学反応によって電子的に励起され、次いで（例えば化学発光計を用いて）測定可能な光を放出する、または蛍光受容体にエネルギーを供与する。酵素標識の例は、ルシフェラーゼ（例えば、ホタルルシフェラーゼ及び細菌ルシフェラーゼ；米国特許第４，７３７，４５６号）、ルシフェリン、２，３－ジヒドロフタラジンジオン、リンゴ酸塩デヒドロゲナーゼ、ウレアーゼ、西洋ワサビペルオキシダーゼ（ＨＲＰＯ）等のペルオキシダーゼ、アルカリホスファターゼ、－ガラクトシダーゼ、グルコアミラーゼ、リソザイム、糖類オキシダーゼ（例えば、グルコースオキシダーゼ、ガラクトースオキシダーゼ、及びグルコース－６－ホスフェートデヒドロゲナーゼ）、ヘテロ環オキシダーゼ（ウリカーゼ及びキサンチンオキシダーゼ等）、ラクトペルオキシダーゼ、ミクロペルオキシダーゼ等を含む。酵素を抗体に抱合させる技術は、Ｏ’Ｓｕｌｌｉｖａｎら，Ｍｅｔｈｏｄｓ ｆｏｒ Ｐｒｅｐａｒａｔｉｏｎ ｏｆ Ｅｎｚｙｍｅ－Ａｎｔｉｂｏｄｙ Ｃｏｎｊｕｇａｔｅｓ ｆｏｒ ｕｓｅ ｉｎ Ｅｎｚｙｍｅ Ｉｍｍｕｎｏａｓｓａｙ，ｉｎ Ｍｅｔｈｏｄｓ ｉｎ Ｅｎｚｙｍ．（編Ｊ．Ｌａｎｇｏｎｅ ＆ Ｈ．Ｖａｎ Ｖｕｎａｋｉｓ）Ａｃａｄｅｍｉｃ ｐｒｅｓｓ，Ｎｅｗ Ｙｏｒｋ，７３：１４７－１６６（１９８１）に記載されている。

【００９７】

酵素－基質の組み合わせは、例えば以下を含む：

（ｉ）西洋ワサビペルオキシダーゼ（ＨＲＰＯ）と基質としての過酸化水素、過酸化水素が染料前駆物質（例えば、オルトフェニレンジアミン（ＯＰＤ）又は３，３’，５，５’－テトラメチルベンジジンヒドロクロリド（ＴＭＢ））を酸化する；

（ｉｉ）アルカリホスファターゼ（ＡＰ）と色素原基質としてのパラ－ニトロフェニルホスフェート；及び

（ｉｉｉ）－Ｄ－ガラクトシダーゼ（β－Ｄ－Ｇａｌ）と色素原基質（例えば、ｐ－ニトロフェニル－Ｄ－ガラクトシダーゼ）又は蛍光原基質４－メチルウンベリフェリル－Ｄ－ガラクトシダーゼ。

当業者には、多くの他の酵素－基質の組み合わせが利用可能である。これらの一般的な概説は、米国特許第４，２７５，１４９号及び第４，３１８，９８０号を参照。

【００９８】

標識は抗体に間接的に抱合されるときもある。当業者は、これを達成するための種々の技術を知っているであろう。例えば、抗体にビオチンを抱合し、上記３つの範疇の標識の任意のものにアビジンを抱合する、又はその逆が可能である。ビオチンはアビジンに選択的に結合し、よってこの間接的な方式で抗体に標識を抱合させることができる。あるいは、

抗体での標識の間接的抱合を達成するために、抗体に小さなハプテン（例えばジゴキシン）を抱合させ、上記した異なる型の標識を抗－ハプテン抗体（例えば抗－ジゴキシン抗体）に抱合させる。よって抗体での標識の間接的抱合が達成できる。
本発明の他の実施態様では、抗－ｔｒｋＣ抗体を標識する必要はなく、その存在が抗－ｔｒｋＣ抗体に結合する標識抗体を用いて検出させる。
本発明の抗体は任意の公知のアッセイ方法、例えば競合結合アッセイ、直接及び間接サンドウィッチアッセイ、及び免疫沈降アッセイ等で用いられうる。Ｚｏｌａ，Ｍｏｎｏｃｌｏｎａｌ Ａｎｔｉｂｏｄｉｅｓ：Ａ Ｍａｎｕａｌ ｏｆ Ｔｅｃｈｎｉｑｕｅｓ，ｐｐ．１４７－１５８（ＣＲＣ Ｐｒｅｓｓ，Ｉｎｃ．１９８７）。

【００９９】

競合結合アッセイは、有限量の抗体との結合における、試験用サンプルに対して競合する標識された標準体の能力による。試験用サンプル中のｔｒｋＣタンパク質の量は、抗体が結合する標準体の量に対して逆比例する。結合する標準体の量の測定を容易にするため、好ましくは抗体は競合の前後に不溶化され、抗体に結合する分析物及び標準体は、便宜上、結合しないで残存する標準体及び分析物から分離する。
サンドイッチアッセイでは、検出されるタンパク質の互いに異なる免疫原部分、又はエピトープ、に結合可能な２つの抗体が使用される。サンドイッチアッセイにおいて、分析されるテスト用サンプルは、固体支持体に固定化された第１の抗体に結合し、その後、第２の抗体が分析物に結合し、よって不溶性の３部位複合体が形成される。例えば、米国特許第４，３７６，１１０号を参照されたい。第２の抗体は検出可能な部分で、それ自身が標識されてもよく（直接サンドイッチアッセイ）、又は検出可能な部分で標識された抗免疫グロブリン抗体を使用して測定してもよい（間接サンドイッチアッセイ）。例えば、一方の種類のサンドイッチアッセイはＥＬＩＳＡアッセイであり、この場合、検出可能な部分は酵素である。

【０１００】

また抗体は、インビボ診断アッセイでも使用できる。一般的に、抗体は放射性核種（^{111}Ｉｎ、^{99}Ｔｃ、^{14}Ｃ、^{131}Ｉ、^{125}Ｉ、^{3}Ｈ、^{32}Ｐ又は^{35}Ｐ等）で標識され、対象の細胞又は組織が免疫シンチオグラフィーによって局在化される。
抗体は、病理学で初発試薬として用いられ、後の技術が当該分野では良く知られている。
本発明の抗－ｔｒｋＣアゴニスト抗体は、向上した効力、向上した薬物動態学的特性（ｐＫ）及び生体利用度、そして投与後の痛覚過敏がないことを含む、治療剤としてＮＴ－３を上回る多くの利点を有すると考えられている。

【０１０１】

４．製薬的組成物

本発明の抗体の治療的製剤は、所望される程度の純度を持つ抗体を凍結乾燥製剤又は水性溶液の形態で、最適な製薬上許容される担体、賦形剤又は安定化剤と混合することにより調製され保存される（Ｒｅｍｉｎｇｔｏｎ’ｓ Ｐｈａｒｍａｃｅｕｔｉｃａｌ Ｓｃｉｅｎｃｅ １６ｔｈ 版，Ｏｓｏｌ，Ａ．編．［１９８０］）。許容される担体、賦形剤、又は安定化剤は、用いられる用量及び濃度で受容者に非毒性であり、リン酸、クエン酸、及び他の有機酸などの緩衝液；アスコルビン酸及びメチオニンを含む酸化防止剤；防腐剤（オクタデシルジメチルベンジルアンモニウムクロライド；ヘキサメトニウムクロライド；ベンズアルコニウムクロライド、ベンズエトニウムクロライド；フェノール、ブチル又はベンジルアルコール；メチル又はプロピルパラベン等のアルキルパラベン；カテコール；レゾルシノール；シクロヘキサノール；３－ペンタノール；及びｍ－クレゾールなど）；低分子量（約１０残基未満）ポリペプチド；血清アルブミン、ゼラチン、又は免疫グロブリン等のタンパク質；ポリビニルピロリドン等の親水性ポリマー；グリシン、グルタミン、アスパラギン、ヒスチジン、アルギニン、又はリシン等のアミノ酸；グルコース、マンノース、又はデキストリンを含む単糖類、二糖類、及び他の炭水化物；ＥＤＴＡ等のキレート剤；スクロース、マンニトール、トレハロース又はソルビトールなどの糖；ナトリウムなどの塩形成対イオン；金属錯体（例えば、Ｚｎ－タンパク質錯体）；及び／又は

トゥイーン（ＴＷＥＥＮ）（商品名）、プルロニクス（ＰＬＵＲＯＮＩＣＳ）（商品名）、及びポリエチレングリコール（ＰＥＧ）等の非イオン性界面活性剤を含む。

【０１０２】

ここでの製剤は、治療すべき特定の徴候に必要な場合に一つ以上の活性化合物、好ましくは互いに悪影響を及ぼさない相補的活性を持つものも含んでよい。これらの分子は、適切には、意図する目的に有効な量の組み合わせで存在する。

また、活性成分は、例えばコアセルベーション技術により又は界面重合により調製されたマイクロカプセル、例えば、各々ヒドロキシメチルセルロース又はゼラチン－マイクロカプセル及びポリ（メタクリル酸メチル）マイクロカプセル中、コロイド状薬物送達系（例えば、リポソーム、アルブミン小球、マイクロエマルション、ナノ粒子及びナノカプセル）中、又はマイクロエマルション中に包括されていてもよい。これらの技術は、Ｒｅｍｉｎｇｔｏｎ’ｓ Ｐｈａｒｍａｃｅｕｔｉｃａｌ Ｓｃｉｅｎｃｅ １６ｔｈ ｅｄｉｔｉｏｎ，Ｏｓｏｌ，Ａ．Ｅｄ．（１９８０）に開示されている。

インビボ投与に使用される製剤は無菌でなければならない。これは、滅菌濾過膜を通した濾過により容易に達成される。

【０１０３】

徐放性製剤を調製してもよい。徐放性製剤の好適な例は、抗体を含有する固体疎水性ポリマーの半透性マトリクスを含み、このマトリクスは成形された物品、例えばフィルム、又はマイクロカプセルの形状である。除放性マトリクスの例は、ポリエステルヒドロゲル（例えば、ポリ（２－ヒドロキシエチル－メタクリレート）又はポリ（ビニルアルコール））、ポリアクチド（米国特許第３，７７３，９１９号）、Ｌ－グルタミン酸及びエチル－Ｌ－グルタメート、非分解性エチレン－酢酸ビニル、ＬＵＰＲＯＮ ＤＥＰＯＴ（商品名）（乳酸－グリコール酸コポリマーと酢酸リュープロリドの注射可能な小球）などの分解性乳酸－グリコール酸コポリマー、ポリ－（Ｄ）－（－）－３－ヒドロキシブチル酸を含む。エチレン－酢酸ビニル及び乳酸－グリコール酸などのポリマーは分子を１００日に渡って放出することができるが、ある種のヒドロゲルはより短時間でタンパク質を放出してしまう。カプセル化された抗体が身体内に長時間残ると、それらは３７℃の水分に露出されることにより変性又は凝集し、その結果、生物学的活性の低下及び起こりうる免疫原性の変化をもたらす。合理的な方法は、含まれる機構に依存する安定化について工夫することができる。例えば、凝集機構がチオ－ジスルフィド交換を通した分子間Ｓ－Ｓ結合形成であると発見された場合、安定化はスルフヒドリル残基の修飾、酸性溶液からの凍結乾燥、水分含有量の制御、適切な添加剤の付加、及び特異的ポリマーマトリクス組成物の開発によって達成されうる。

【０１０４】

治療的に用いられる抗体の有効量は、例えば、治療目的、投与経路、及び患者の状態に依存する。従って、治療者は、最大の治療効果を得ることが要求されるときに、用量を滴定し投与経路を修正する必要があるであろう。典型的な用量は、上記の因子に依存し、約１ｇ／ｋｇから１００ｍｇ／ｋｇ又はそれ以上の範囲であろう。典型的には、臨床医は、上記の疾患の治療についての所望の効果が達成される用量に達するまでアンタゴニストを投与するであろう。この治療の進展は、従来のアッセイによって容易にモニターされる。

投与は、限定されることなく、静脈、皮下、局所、気管内、鼻腔内、肺内、及びｉｎｔｒａｐａｒｙｎｃａｌ投与を含む当該分野で知られている任意の従来型の経路であり得る。

【０１０５】

４．遺伝子治療

本発明の抗体をコードする核酸は、種々（慢性）の神経変性疾患及び急性神経細胞損傷、特に遺伝的に獲得された神経障害の遺伝子治療にも用いられ得る。遺伝子治療への二つの基本的手法が発展している：エキソビボ遺伝子治療及びインビボ遺伝子治療である。エキソビボ遺伝子治療では、細胞を被検体から取り出してインビボで培養する。機能的な置換遺伝子をインビボで細胞へ導入し、この修飾細胞を培養で増し、次に被検体へ再移植する。インビボ遺伝子治療では、標的遺伝子を被検体から取り出さない。むしろ、転移遺伝子

をインサイツで受容体の細胞、すなわち受容体内へ導入する。
ヒトにおける幾つかのエキソビボ遺伝子治療が報告され、例えば、Ａｎｄｅｒｓｏｎ，Ｓｃｉｅｎｃｅ ２５６：８０８－８１３（１９９２），及びＭｉｌｌｅｒ，Ｎａｔｕｒｅ ３５７： ４５５－４６０（１９９２）で報告されている。
【０１０６】
インビボ遺伝子治療の生存度は、Ｆｅｌｇｎｅｒら．，Ｎａｔｕｒｅ ３４９： ３５１－３５２（１９９１）に概説されているように、幾つかの動物モデルで示されている。直接の遺伝子移転は、例えば、筋肉組織（Ｆｅｒｒｙら．，Ｐｒｏｃ．Ｎａｔｌ．Ａｃａｄ．Ｓｃｉ．８８： ８３７７－８７８１［１９９１］；Ｑｕａｎｔｉｎら．，Ｐｒｏｃ．Ｎａｔｌ．Ｓｃｉ．ＵＳＡ ８９： ２５８１－２５８４［１９９２］）；動脈壁（Ｎａｂｅｌら．，Ｓｃｉｅｎｃｅ ２４４： １３４２－１３４４［１９８９］）；そして神経系（Ｐｒｉｃｅら．，Ｐｒｏｃ．Ｎａｔｌ．Ａｃａｄ．Ｓｃｉ．８４： １５６－１６０［１９８７］）で報告されている。
従って、本発明は、アゴニスト抗－ｔｒｋＣ抗体をコードするポリヌクレオチドの細胞への送達に適した送達媒体も提供する（インビボ又はエキソビボに関わらず）。一般的に、抗体（例えば、直鎖状抗体又は抗体鎖）をコードするポリヌクレオチドは、プロモーター及び異種ポリヌクレオチドと作用可能に連結している。宿主細胞への導入に関して、本発明の抗体をコードするポリヌクレオチドの取り込みに適した送達媒体には、非ウイルス媒体及びウイルスベクターが含まれる。Ｖｅｒｍａ及びＳｏｍｉａ，Ｎａｔｕｒｅ ３８９： ２３９－２４２（１９９７）。
【０１０７】
本発明の抗体をコードするポリヌクレオチドの送達に関する幅広い種々の非ウイルス媒体は当該分野で知られており、本発明に包含されている。抗－ｔｒｋＣ抗体をコードするポリヌクレオチドは、むき出しのＤＮＡとして細胞へ送達することができる（米国特許第５，６９２，６２２号；国際公開 ９７／４０１６３）。或いは、ここでの抗－ｔｒｋＣ抗体をコードするポリヌクレオチドは、カチオン性脂質に限定されないが；天然ポリマー及び合成ポリマーを含む生物適合性ポリマー；脂質タンパク質；ポリペプチド；多糖類；脂質多糖類；人工ウイルス外皮；金属粒子；及び細菌を含む種々の物質（送達の形態）と種々の方法で関連する細胞へ送達が可能である。送達媒体を、微粒子とすることができる。これらの種々の物質の混合物又はコンジュゲートは、送達媒体としても用いることができる。ここでの抗体をコードするポリヌクレオチドは、これら種々の送達の形態と非共有又は共有的に結合することができる。リポソームは、特定の細胞型、例えば腎糸球体上皮細胞を標的とすることができる。
【０１０８】
限定されないが、ウイルスベクターには、例えばアデノウイルス、単純ヘルペスウイルス、ワクシニア・ウイルスのようなポックス・ウイルス、及びアデノ随伴ウイルスを含むパルボウイルスに基づくＤＮＡウイルスベクター；そして限定されないがレトロウイルスベクターを含むＲＮＡウイルスベクターが含まれる。レトロウイルスベクターには、マウス白血病ウイルスを含むレトロウイルスベクター、そしてヒト免疫欠損症などのレンチウイルスが含まれる。Ｎａｌｄｉｎｉら．，Ｓｃｉｅｎｃｅ ２７２： ２６３－２６７（１９９６）。
抗－ｔｒｋＣ抗体をコードするポリヌクレオチドを含む非ウイルス送達媒体は、例えば、リン酸カルシウム共沈殿技術によるトランスフェクション；エレクトロポレーション；電気透過化処理；リポソーム媒介トランスフェクション；弾道トランスフェクション、微粒子銃トランスフェクション；微粒子照射を含む微粒子銃プロセス、ジェット注入、そして針及び注射器注入；又は微量注入法による、当該分野にて既知の任意の方法によって宿主細胞及び／又は標的細胞へ導入することができる。トランスフェクションの多くの方法が、この分野の熟練研究者に知られている。
ウイルス送達媒体は、感染によって細胞へ導入することができる。或いは、ウイルス媒体を、細胞への送達のために、上記の任意の非ウイルス送達媒体へ取り込ませることができ

る。例えば、ウイルスベクターを、カチオン性脂質（Ｈｏｄｇｓｏｎ及びＳｏｌａｉｍａｎ，Ｎａｔｕｒｅ Ｂｉｏｔｅｃｈｎｏｌ．１４：３３９－３４２［１９９６］）；又は層状リポソーム（Ｗｉｌｓｏｎら．，Ｐｒｏｃ．Ｎａｔｌ．Ａｃａｄ．Ｓｃｉ．ＵＳＡ７４：３４７１［１９７７］；及びＦａｌｌｅｒら．Ｊ．Ｖｉｒｏｌ．４９：２６９［１９８４］）と混合することができる。

【０１０９】

好ましい実施態様では、本発明の抗－ｔｒｋＣ抗体の重鎖及び軽鎖（断片を含む）の双方をコードする核酸は、米国特許第４，９６５，１９６号及び４，７１３，３３９号に開示されたような同じポリシストロニック発現ベクターに存在する。ポリシストロニック発現ベクターは、二次タンパク質及び所望されるタンパク質をコードする配列を含み、その所望及び二次配列の双方は同じプロモーターによって支配されている。このコード化配列は、翻訳終止及び開始シグナルコドンによって分離されている。二次配列の発現は、所望するタンパク質の配列の発現に関するコントロールへ作用し、二次タンパク質は、トランスフェクト細胞の選択のためのマーカーとして機能する。

インビボの遺伝子治療では、例えば、ベクターは静脈注射（ｉ．ｖ．）によって受容体へ投与され得る。適切な力価は、種々の因子、例えば選択された特定のベクター、宿主、用いられるプロモーターの強度、そして治療された疾患の重症度に依存する。

【０１１０】

本発明は、以下の限定されない実施例によって、さらに例示される。

【０１１１】

実施例

実施例１：　アゴニスト抗－ｔｒｋＣモノクローナル抗体の生産と特徴付け

抗体の生産及びアイソタイピング

ヒトＩｇＧ２又はＩｇＧ４（Ｘｅｎｏｍｉｃｅ、Ｍｅｎｄｅｚら．，Ｎａｔｕｒｅ Ｇｅｎｅｔｉｃｓ １５：１４６－１５６［１９９７］に記載）を生産するトランスジェニックマウス及び野生型Ｂａｌｂ／Ｃマウスは、Ｍｅｎｄｅｚら．（上掲）に記載のようにフロインド又はリビアジュバントのいずれかの中の２０μｇのヒトｔｒｋＣ－ＩｇＧ（Ｓｈｅｌｔｏｎら．，Ｊ．Ｎｅｕｒｏｓｃｉ．１５：４７７－４９１［１９９５］）で、後部足蹠を通して腹腔内に、又は皮下のどちらかで超免疫化した。免疫マウスからの脾臓細胞を、メラノーマ細胞（Ｘ６３．Ａｇ８．６５３，ＡＴＣＣ ロックビル，メリーランド）と融合させた。合計３３の融合を、２５３のＸｅｎｏｍｉｃｅ及び３５の野生型Ｂａｌｂ／Ｃマウスを用いておこなった。プレート（計２１，７３４ウェル）を、ｔｒｋＣ－ＩｇＧを用いて直接ＥＬＩＳＡによって最初にスクリーニングした。ＥＬＩＳＡスクリーニングによって、６８４ｔｒｋＣポジティブハイブリドーマを同定し、次にその全てを、ｔｒｋＣ ＫＩＲＡ（キナーゼ活性化レセプターアッセイ）でのアゴニスト活性について評価した。ＫＩＲＡによって、抗－ｔｒｋＣアゴニスト抗体を分泌する、１４のＸｅｎｏｍｏｕｓｅ派生、及び２２の野生型Ｂａｌｂ／Ｃマウス派生ハイブリドーマを同定した。これらのハイブリドーマを限界希釈法によってサブクローニングし、アゴニスト活性を確認するために再アッセイし、そしてプリスタンで初回刺激したＢａｌｂ／Ｃ又はヌードマウス（Ｈｏｎｇｏら．，Ｈｙｂｒｉｄｏｍａ １４：２５３－２６０［１９９５］）へ注入することによって腹水症を誘導するために用いた。腹水に存在するモノクローナル抗体を、プロテインＡアフィニティークロマトグラフィーによって精製した（Ｈｏｎｇｏら．，上掲）。特異的融合効率（ポジティブの数／スクリーニングされたウェルの数）は、Ｘｅｎｏｍｏｕｓｅ及び野生型Ｂａｌｂ／Ｃマウス融合物の双方で３％であった。アゴニストモノクローナル抗体の出現率（アゴニスト／ｔｒｋＣ ＥＬＩＳＡポジティブの数）は、Ｘｅｎｏｍｏｕｓｅ及び野生型Ｂａｌｂ／Ｃマウス融合物のそれぞれで３％及び８％であった。マウスモノクローナル抗体のアイソタイプは、提供者の指示書に従って、ＧＩＢＣＯ ＢＲＬの計深棒又はＺｙｍｅｄ マウスータイパー イソタイピング キットのいずれかを用いて測定した。Ｘｅｎｏｍｉｃｅは、IgG_2 又は IgG_4 系統のいずれかで、対応する抗体のアイソタイプを生産した。表１は、種々のヒト及びマウス抗－ｔｒｋＣ

モノクローナル抗体のアイソタイプを示す。合計８のヒトＩｇＧ$_2$、６のヒトＩｇＧ$_4$、７のマウスＩｇＧ$_1$、１０のマウスＩｇＧ$_{2a}$及び５のマウスＩｇＧ$_{2b}$モノクローナル抗体が同定された。ＫＩＲＡアッセイで測定された、最も強力なアゴニスト活性を有するモノクローナル抗体（表２では、星印で示す）は、詳細な特徴付けで選択された。

【０１１２】

表２

ヒトＭａｂｓ（計１４）

ＩｇＧ$_2$アイソタイプ（8Ｍａｂｓ）	ＩｇＧ$_4$アイソタイプ（6Ｍａｂｓ）
２．５．１*	４．８
６．１．２*	２３３７
６．４．１*	２３３８
２３４２	２３３９
２３４３	２３４８
２３４４*	２３４９
２３４５*	
２３４６	

マウス Ｍａｂｓ（計２２）

ＩｇＧ$_1$ (7)	ＩｇＧ$_{2a}$ (１０)	ＩｇＧ$_{2b}$ (５)	ＩｇＧ$_3$
２２４９	２２４８*	２２５２	
２２５０*	２２７２	２２７３	
２２５３*	２２５１	２２７７	
２２５４	２２５５	２２７９	
２２５６*	２２７４	２２８０	
２２５７	２２７５		
２２６０	２２７６		
	２２７８		
	２２８１		
	２２８２		

【０１１３】

アゴニスト活性の決定

ａ．ＫＩＲＡアッセイ

抗－ｔｒｋＣモノクローナル抗体のＮＴ－３アゴニスト活性を決定するために、二つのバイオアッセイを利用した。上記にてかなり詳しく論じられているキナーゼ活性化レセプターアッセイ（ＫＩＲＡ）は、例えばＮＴ－３又はアゴニストモノクローナル抗体のようなリガンドによる刺激への応答における、トランスフェクト細胞のｔｒｋＣのチロシンリン酸化を測定する（Ｓａｄｉｃｋら．，Ｅｘｐ．Ｃｅｌｌ Ｒｅｓ．２３４：３５４－３６１［１９９７］）。モノクローナル抗体を、ＫＩＲＡ刺激バッファー（２％ウシ血清アルブミン［ＢＳＡ，Ｉｎｔｅｒｇｅｎ Ｃｏ．，Ｐｕｒｃｈａｓｅ，ＮＹ］及び２５ｍＭ Ｈｅｐｅｓを含む、０．２μｍの濾過されたＦ１２／ＤＭＥＭ ５０：５０）で２７μｇ／ｍｌに希釈した。モノクローナル抗体を、さらに、刺激培地で１：３（全体で８希釈；濃度範囲は０．０１－１８０ｎＭ）に希釈した。ＨＳＶグリコ糖タンパク質 Ｄ（ｇＤ）から誘導された２６アミノ酸ポリペプチドフラッグエピトープと融合したｔｒｋＣで安定にトランスフェクトしたチャイニーズ・ハムスター・卵巣（ＣＨＯ）細胞を、９６－ウェル細胞培養プレートに播種（５ｘ１０^{4}細胞／ウェル）し、生育させた。次いで、この細胞を、０．１；１．５６；３．１３；６．２５；１２．５；２５；５０及び１００ｎｇ／ｍｌの連続希釈によって、ＮＴ－３（ポジティブコントロールとして）又は種々の抗－ｔｒｋＣモノクローナル抗体のいずれかで刺激した。すべての希釈は、６時間にわた

って重複してアッセイした。このアッセイは、Ｓａｄｉｃｋら．，（上掲）に記載した通りに実行した。要約すると、細胞をＴｒｉｔｏｎ Ｘ－１００を用いて可溶化し、可溶化物に存在するｔｒｋＣを、ｇＤエピトープに対する抗体を用いてＥＬＩＳＡで捕獲し、酵素と安定に共役した抗－ホスホチロシン抗体を用いてリン酸化ｔｒｋＣを検出して定量化した。ｔｒｋＣに対するものではないモノクローナル抗体（抗－ＩＬ８ IgG_2 Ｘｅｎｏｍｏｕｓｅ誘導ヒト抗体又は抗－ｇｐ１２０ IgG_1 マウスモノクローナル抗体）を、ネガティブコントロールとして用いた。図１（Ａ及びＢ）に示されているように、すべての選択された抗－ｔｒｋＣモノクローナル抗体は、それらがｔｒｋＣレセプターのチロシンリン酸化を刺激できる限りは、ＮＴ－３の活性を模倣することができる。ヒト抗－ｔｒｋＣモノクローナル抗体（図１Ａ）は、マウス抗－ｔｒｋＣモノクローナル抗体よりさらに強力なアゴニスト活性を示した（図１Ｂ）。例えば、最も良いヒト抗－ｔｒｋＣモノクローナル抗体は、最も良いマウス抗－ｔｒｋＣモノクローナル抗体よりも１０倍さらに強力である。さらには、ヒトモノクローナルは、特により低い範囲の濃度で、ＮＴ－３とほぼ同じように有効である。

【０１１４】

ｂ．ＰＣ１２神経突起増殖アッセイ

抗－ｔｒｋＣモノクローナル抗体のＮＴ－３模倣活性を測定するために用いられるその他のアッセイは、ＰＣ１２神経突起増殖アッセイであった。このアッセイは、適切なリガンドによる刺激に応答するラット フェォチトクローマ細胞（ＰＣ１２）による、神経突起プロセスの増殖を測定する。これらの細胞は内因性のｔｒｋＡを発現するので、ＮＧＦに対して応答性がある。しかしながら、それらは内因性のｔｒｋＣを発現しないので、ＮＴ－３及びそのアゴニストに対する応答を引き出すためには、ｔｒｋＣ発現構成物でトランスフェクトされる。ＰＣ１２細胞は、完全長ヒトｔｒｋＣでトランスフェクトされ（Ｕｒｆｅｒら．，Ｂｉｏｃｈｅｍ．３６：４７７５－４７８１［１９９７］；Ｔｓｏｕｌｆａｓら．，Ｎｅｕｒｏｎ １０：９７５－９９０［１９９３］）、９６ウェルの細胞培養プレートにプレートされた（１０００細胞／ウェル）。トランスフェクションの３日後、抗－ｔｒｋＣモノクローナル抗体を三重に添加し（０．０００２から２．７ｎＭの濃度範囲）、３７℃でさらに３日間にわたってインキュベートした。次いで、細胞を位相差顕微鏡法で分析し、細胞の直径の２倍を超える神経突起を有する細胞を数えた。マウス抗－ｔｒｋＣモノクローナル抗体と同じくヒトは、図１Ｃ及びＤに示すように、ＰＣ１２細胞での神経突起の増殖を刺激する。ヒト抗－ｔｒｋＣモノクローナル抗体（図１Ｃ）は、マウス抗－ｔｒｋＣモノクローナル抗体（図１Ｄ）よりもさらに強力な活性を示し、それによってＫＩＲＡアッセイで得られた結果が実証された。さらには、ＫＩＲＡアッセイの結果と一致して、ヒト抗－ｔｒｋＣモノクローナル抗体は、ＮＴ－３で得られたのとほぼ類似の刺激を示した。上記の二つのバイオアッセイで得られた結果は、ｔｒｋＣレセプターの天然リガンドである、ＮＴ－３の活性を模倣する抗－ｔｒｋＣモノクローナル抗体の能力を示す。

【０１１５】

モノクローナル抗体のアゴニスト活性は、チロシンリン酸化の最大誘導、及びＫＩＲＡアッセイでのリン酸化曲線の算出ＥＣ５０、そしてＰＣ１２神経突起増殖アッセイによって評価される。表３は、種々の抗－ｔｒｋＣアゴニストモノクローナル抗体の特徴を要約している。

【０１１６】

表３

Mab ID	アイソタイプ	アゴニスト活性 KIRA/PC12	ラット trkC との結合	イムノブロット NR/Red.	親和性 Kd(nM)
ヒト Mabs				NR Red.	
2.5.1	G2	(+++/+++)	NO	++ ++	12
6.1.2	G2	(++++/++++)	NO	+ +	12.5
6.4.1	G2	(++++/++++)	YES	+ +	12
2344	G2	(+++/+++)	NO	++ +	19
2345	G2	(++++/++++)	NO	++ ++	12.1
2349	G4	(++++/++++)	NO	++ +	23
マウス Mabs					
2248	G2a	(+/+)	NO	+++ -	5.9
2250	G1	(++/++)	NO	++ ++	8.7
2253	G1	(++/+)	NO	++ ++	42
2256	G1	(+/+)	YES	++ +	62

【０１１７】

抗－ｔｒｋＣ抗体の試験特異性

抗－ｔｒｋＣモノクローナル抗体の特異性は、直接ＥＬＩＳＡを用いて試験された。マイクロタイタープレートを、５０ｍＭ 炭酸塩バッファー、ｐＨ９．５で希釈した１００μｌの１μｌ／ｍｌ溶液を用いたレセプターｔｒｋＡ－ＩｇＧ、ｔｒｋＢ－ＩｇＧ又はｔｒｋＣ－ＩｇＧを捕獲抗原（Ｓｈｅｌｔｏｎら．，Ｊ．Ｎｅｕｒｏｓｃｉ．１５：４７７－４９１［１９９５］）とした免疫アドヘシン構成物で終夜にわたってコーティングした。ＣＤ４－ＩｇＧ（Ｃａｐｏｎら．，Ｎａｔｕｒｅ ３３７：５２５－５３１［１９８９］）を捕獲抗原に置き換えてネガティブコントロールとして用いた。コーティングしたプレートを、０．５％ＢＳＡ及び０．０５％Ｔｗｅｅｎ２０を含むＰＢＳで希釈した種々の濃度の抗－ｔｒｋＣモノクローナル抗体（１００μｌの０．０１から１μｇ／ｍｌ）とともに室温で１時間にわたってインキュベートした。過度の非結合抗体を除くための洗浄の後に、適切なＨＲＰコンジュゲート（ヒトモノクローナル抗体：ヤギ抗－ヒトκ－ＨＲＰ、１：５０００希釈；マウスモノクローナル抗体：ヤギ抗－マウスＩｇＧ（Ｆｃ）－ＨＲＰ、１：５０００希釈）を添加し、室温で１時間にわたってインキュベートした。次いで、プレートを洗浄し、前出に記載のように展開して読みとった（Ｈｏｎｇｏら．，Ｈｙｂｒｉｄｏｍａ １４：２５３－２６０［１９９５］）。図２は、ヒト抗－ｔｒｋＣモノクローナル抗体６．１．２を用いた代表的な実施例を示す。結合は、ｔｒｋＣに対してより高く特異性があり、ｔｒｋＡ又はｔｒｋＢのいずれとも顕著な交差反応が観察されなかった。同じように、他のヒト及びマウス抗－ｔｒｋＣモノクローナル抗体は、ｔｒｋＣの特異的認識を示した。

【０１１８】

ヒトｔｒｋＣに対して用いられた捕獲抗原がｔｒｋＣ－ＩｇＧではなくｔｒｋＣ－ｇＤであったことを除いて、ヒトｔｒｋＣ及びラットｔｒｋＣに対する種々の抗－ｔｒｋＣアゴニストモノクローナル抗体の結合を、基本的に上記の直接ＥＬＩＳＡを用いて比較した。図３に示した結果は、ヒト抗－ｔｒｋＣモノクローナル抗体の中で、６．４．１のみが顕著にラットｔｒｋＣを認識し、残りはヒトｔｒｋＣに対して特異的であったことを示す。

同じように、マウスモノクローナル抗体の中では、他がヒトｔｒｋＣのみへの特異的認識をを示した一方で、２２５６のみがラットｔｒｋＣを顕著な程に認識した。

【０１１９】

親和性研究

抗－ｔｒｋＣアゴニストモノクローナル抗体の親和性は、ＢＩＡｃｏｒｅ－２０００（商品名）表面プラズモン共鳴装置（ＳＰＲ）システム（ＢＩＡｃｏｒｅ，Ｉｎｃ．，Ｐｉｓｃａｔａｗａｙ，Ｎ．Ｊ．）を用いて測定された。ＣＭ５バイオセンサーチップを、提供者の指示書に従ってＮ－エチル－Ｎ’－（３－ジメチルアミノプロピル）－カルボジイミド塩酸塩（ＥＤＣ）及びＮ－ヒドロキシスクシニミド（ＮＨＳ）で活性化した。結合実験の最初のシリーズでは、抗原、ｇＤ－タッグｔｒｋＣを１０ｍＭ 酢酸ナトリウムバッファー（ｐＨ４．８）で希釈し、０．０９ｍｇ／ｍＬの濃度で活性化チップに注入した。変動照射時間によって、４つの抗原密度範囲が達成された：１４，０００－１７，０００レスポンスユニット（ＲＵ）、７０００－９０００ＲＵ、２０００－３０００ＲＵ、そして４００－６００ＲＵ。このチップをエタノールアミンでブロックした。

反応速度測定の最初のシリーズでは、抗－ｔｒｋＣ抗体（ＩｇＧ’ｓ）をランニングバッファー（０．０５％ Ｔｗｅｅｎ－２０及び０．０１％ アジ化ナトリウムを含むＰＢＳ）で希釈し、０．０３ｍｌ（６６７ｎＭ）を０．０１ｍｌ／分の流速の２５℃でバイオセンサーチップに注入した。再生は、１０ｍＭ ＨＣｌの３０秒パルス、それに続く１００ｍＭ Ｔｒｉｓ－ＨＣｌ、ｐＨ８．０の１分のパルス及び二回の洗浄段階で達成された。

実験の第二番目のシリーズでは、抗体密度が１０００－２０００ＲＵに限定されていることを除いて、ＩｇＧ’ｓ（１０ｍＭ 酢酸ナトリウム中で０．１ｍｇ／ｍＬ，ｐＨ４．８）を上記のように固定化した。次に、上記のような反応速度測定のために、３．７Ｍから２９ｎＭの範囲のｇＤ－ｔｒｋＣの２倍連続希釈液をバイオセンサーチップへ注入した。

【０１２０】

各動力学的曲線の解離フェーズは、単一指数解離速度（k_{off}）に適合し、これら速度は、単純な１：１のラングミュア結合モデルによって、注入フェーズの結合速度（k_{on}）の計算に用いられた（Ｌｏｆａｓ ＆ Ｊｏｈｎｓｓｏｎ，１９９０）。

ＳＰＲ測定による平衡解離定数、K_dは、比率$k_{off}／k_{on}$のように計算された。

ｇＤ－ｔｒｋＣに対する抗－ｔｒｋＣ抗体の親和性は、固定化された抗原又は抗体のいずれかによるＳＰＲ動力学実験で測定された。低い密度の固定化抗原（０．４から０．６ｎｇ／ｍｍ２）を用いた実験から確定した見かけ上の親和性は、固定化ＩｇＧを用いた実験で確定したそれらと通常は一致する（図２参照）。しかしながら、固定化ｇＤ－ｔｒｋＣのより高い密度では、恐らくは二価ＩｇＧによる結合のアビディティ効果のために、各抗体の見かけ上の結合親和性は、１０倍ほどの因子によって次第に堅固となる（データは示されていない）。ある場合には、ｔｒｋＣが固定化ＩｇＧへ注入された場合、結合は検出されない。これは、ＩｇＧの固定化が抗原結合部位の立体的な遮断につながるために生じる。すべての条件が試験された下で、抗体２２４８は、すべての試験された抗体の中で、最も高い見かけ上の親和性（K_d＝５．６から８．５ｎＭ）を有した。

【０１２１】

表４

ＳＰＲによって測定した結合親和性。固定化ｇＤ-ｔｒｋＣ（４００-６００ ＲＵ）へのＩｇＧの結合、及び固定化ＩｇＧ（１０００-３０００ ＲＵ）へのｇＤ-ｔｒｋＣの結合に関する結果を示す。ＮＤＢ＝検出できない結合。

	K_d(nM)	
抗体（IgG）	固定化 gD-trkC	固定化 IgG
2248	5.9	8.5
2250	8.7	28
2253	42	51
2256	62	300
2344	19	NDB
2345	12	NDB
2349	23	NDB
6.4.1	12	28
6.1.2	13	16
2.5.1	12	NDB

【０１２２】

競合ＥＬＩＳＡ

抗体が認識するｔｒｋＣ上のエピトープに基づいた、これら抗体が属する種々のグループに関する予備情報を得るために、競合ＥＬＩＳＡを利用した。このアッセイでは、ｔｒｋＣ－ｇＤ（１μｇ／ｍｌ）を、マイクロタイタープレートをコートする捕獲抗原として利用した。特異的なビオチン化抗－ｔｒｋＣモノクローナル抗体（１μｇ／ｍｌ）を、それのみ又は非標識であって標識抗体と比較して多く（５０μｇ／ｍｌ）用いられたその他の抗－ｔｒｋＣモノクローナル抗体の存在下でコーティングしたプレートへ添加した。ビオチン化抗体及び非標識抗体の双方が同じ又は重なり合うエピトープを認識するならば、それらは、固定化ｔｒｋＣとの結合に関して競争し、標識抗体の結合が減少する結果となる。それらが異なっていて重なり合っていないエピトープを認識するならば、それらの間には競争がなく、固定化ｔｒｋＣと標識抗体の結合は影響されない。非標識ヒトＩｇＧ２及びマウスＩｇＧは、ネガティブコントロールとして用いられる。図４の代表的データは、マウス抗－ｔｒｋＣ２２４８モノクローナル抗体を除くすべての抗－ｔｒｋＣモノクローナル抗体が、固定化ｔｒｋＣとの結合に関して、標識ヒト抗－ｔｒｋＣ６．１．２モノクローナル抗体と競争することを示し、マウス２２４８抗体が、すべての他の抗－ｔｒｋＣ抗体によって認識されるエピトープとは異なったｔｒｋＣ上のエピトープを認識することを示唆している。

【０１２３】

非標識マウスモノクローナル抗体２２４８が最初に固定化ｔｒｋＣと結合する場合に、他の（ビオチン化）抗体のどれもが、それらの結合部位に接近することができず、そのことがエピトープが明確でも、立体的妨害が役割を担うことを示唆していることに注視することは興味あることである。そのようなペアを成す比較は、価値ある情報を示し、同じ抗原に対する抗体をエピトープ認識に基づいて異なるグループへ分類することに役立つ。そのような比較の要約が図５に示されている。この結果は、抗体が二つの明確なグループへ分けることができることを示す：グループ１は、２２４８を除くすべてのモノクローナル抗体を包含し、その一方で、グループ２は、２２４８で構成される。

【０１２４】

ドメイン交換変異をともなうエピトープマッピング

さらなるエピトープマッピングを、種々のドメインがｔｒｋＡ又はｔｒｋＢの対応するドメインと置き換わったキメラｔｒｋＣを用いておこなった。この方法は、抗－ｔｒｋＣ抗体がｔｒｋＡ又はｔｒｋＢと顕著に交差反応をしないという事実によって、可能になった。そのようなドメイン交換変異の用途は、欠損変異に関して明かな利点を有する。ドメイン交換変異体における、ドメインの、関連タンパク質からの類似の大きさ及び実質上は類似のアミノ酸配列である相当するドメインによる置換は、二次構造を保持するようであるが、ドメインの欠失は、タンパク質の二次構造を破壊し得る。ｔｒｋレセプターの細胞外ドメインは、図６Ａに示すように５つのドメインで構成される。Ｄ１及びＤ３はシステイン－リッチドメインであり、Ｄ２はロイシン－リッチドメイン、そしてＤ４及びＤ５は免疫グロブリン様ドメインである。ｔｒｋＢ又はｔｒｋＡのからの対応するドメインで置き換えたＤ１、Ｄ４及びＤ５を含むｔｒｋＣのドメイン交換変異体を作成した（Ｕｒｆｅｒら．，ＥＭＢＯ Ｊ．１４：２７９５－２８０５［１９９５］）。野生型ｔｒｋＣ及び野生型ｔｒｋＡは、それぞれポジティブ及びネガティブコントロールとして用いられた。ｔｒｋＣのドメイン交換変異体は、置換されたドメインのソースによって命名された。例えば、ｓ１ＢはｔｒｋＢからのＤ１ドメインを有し、ｓ４ＢはｔｒｋＢからのＤ４ドメインを有し、ｓ５ＢはｔｒｋＢからのＤ５ドメインを有し、そしてｓ５ＡはｔｒｋＡからのＤ５ドメインを有する。すべての変異体は、免疫アドヘシン、すなわちＩｇＧとの融合で精製されて発現された。

【０１２５】

種々のドメイン交換変異体との各アゴニスト抗－ｔｒｋＣモノクローナル抗体の結合は、ＥＬＩＳＡによって評価される。ヤギ抗－ヒトＩｇＧからのＦ（ａｂ’）$_2$断片は、マイクロタイタープレートをコーティングし、免疫アドヘシン（免疫アドヘシンとしてのｔｒｋＣ－ＩｇＧ、ｔｒｋＡ－ＩｇＧ及びｔｒｋＣのドメイン交換変異体）の連続希釈（１００μｇ／ｍｌから２．４ｎｇ／ｍｌ、１００μｌ／ウェル、室温で１時間）を捕獲するために用いられた。非標識ヒト又はビオチン化マウス抗－ｔｒｋＣモノクローナル抗体のいずれかを、固定化免疫アドヘシンを含むプレートへ添加（ウェル当たり１００μｌ、室温で１時間）し、結合していない過度の試薬を取り除くために洗浄し、そしてヤギ抗－ヒトκ又はＨＲＰと共役させたストレプトアビジンでインキュベートした。図６Ｂに示されているように、すべてのヒト抗－ｔｒｋＣモノクローナル抗体は、ドメインＤ１又はＤ４の置換を有するｔｒｋＣドメイン交換変異体を認識することができる。しかしながら、ｔｒｋＢ又はｔｒｋＡのいずれかから誘導した対応するドメインで置換したドメインＤ５は、抗－ｔｒｋＣ抗体による認識を破壊した。結合の程度は、ネガティブコントロールであるｔｒｋＡで観察されたような同じ低いレベルへ減少した。この結果は、試験されたすべてのヒト抗－ｔｒｋＣモノクローナル抗体が、ドメインＤ５のどこかに位置するエピトープを認識することを示唆している。

【０１２６】

利用された二次抗体がＨＲＰとカップリングしたヤギ抗－マウスＩｇＧ Ｆｃであったことを除いて、類似の分析が、マウス抗－ｔｒｋＣモノクローナル抗体によって基本的に同じ方法でおこなわれた。ヒト抗－ｔｒｋＣ抗体でのように、ドメインＤ５の置換は、試験されたすべてのマウス抗－ｔｒｋＣモノクローナル抗体との結合を破壊する（図６Ｂ）。さらには、ドメインＤ４の置換も、２２４８マウス抗－ｔｒｋＣ抗体の結合を破壊する。マウス抗－ｔｒｋＣアゴニストモノクローナル抗体と同様にヒトは、２２４８マウス抗体を除いて、すべてドメイン５のエピトープを認識すると思われ、さらにはドメイン４の決定基を認識すると思われる。２２４８は、ドメイン４及び５と重なり合う直鎖状のエピトープであり得る。或いは、２２４８抗体は、ドメイン４及びドメイン５の双方から誘導された決定基を有する不連続エピトープによって形成される二次構造を認識し得る。興味あることに、Ｕｒｆｅｒら．（Ｊ．Ｂｉｏｌ．Ｃｈｅｍ．２７３：５８２９－５８４０［１９９８］）は、ＮＴ－３との相互作用を媒介することに関する、ｔｒｋＣレセプターのドメイン５の重要な役割をより早く立証していた。驚くべきことは、ここで記載の抗体も、ＮＴ－３によって認識されたものと大きく重なり合うｔｒｋＣのエピトープと結合す

る。これは、ＮＴ－３及び免疫グロブリン分子の相対的な大きさ及び形状を考えると、驚くべきことである。これらアクチベーターの作用の類似の様式は、二つのｔｒｋＣ分子の細胞外ドメインを架橋させて、それらの細胞内チロシンキナーゼドメインを会合させ、そしてクロスリン酸化してそれらを活性化させることである。

【０１２７】

ホモ二量体ＮＴ－３では、ｔｒｋＣと相互作用する分子の二つの領域が、互いから１８０℃離れている、反対側の分子と正反対にあることが立証された。これらの領域の間の距離は、およそ１６Åである。一方では、ここで記載の免疫グロブリン分子の２つのｔｒｋＣ相互作用部位は、全く正反対ではない。ＮＴ－３よりも異なる角度でｔｒｋＣ結合ドメインを表示することに加えて、免疫グロブリンは、ＮＴ－３でよりももっと広い距離でそれぞれが離れているｔｒｋＣ結合ドメインを有する。これは、５０Åから１５０Åの範囲であるが、２つのＦａｂドメインの正確な角度で変化する。ｔｒｋＣ上の同じ部位に結合した場合には、ＮＴ－３及びアゴニストＭａｂｓのような２つのそのような非常に異なるクロスリンカーがアゴニストとして作用することを予測することは困難であった。

【０１２８】

部位特異的突然変異誘発

部位特異的突然変異誘発法は、抗－ｔｒｋＣ抗体による認識での、選択されたドメイン５の個々のアミノ酸残基の貢献を確定するために用いられる。図７は、ヒトｔｒｋＣドメイン４及び５のアミノ酸配列を示す。それぞれＥ，Ｈ，及びＫに変えられた残基Ｌ２８４，Ｌ２８６及びＥ２８７を除いて、すべての点付き残基をアラニンへ突然変異した（Ｕｒｆｅｒら．，Ｊ．Ｂｉｏｌ．Ｃｈｅｍ．２７３：５８２９－５８４０［１９９８］）。合計で２６の単一のアミノ酸変異が作成され、抗－ｔｒｋＣモノクローナル抗体との結合のそれらの効果に関して評価した。表５に示されている値は、変異体対野生型ｔｒｋＣの抗－ｔｒｋＣ抗体との結合の比率を示している。変動を最小にして効果的な比較を示すために、各抗体に対する各変異体について、ＥＣ５０値を確定し、それを野生型ｔｒｋＣで得たＥＣ５０値で割った。

【０１２９】

表5

trkC変異体	NT-3*	2.5.1	6.1.2	6.4.1	2344	2345	2349	2248	2250	2253	2256	1436
trkC	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
R275A	1.1											
E280A	0.7											
E283A	1.7											
L284E	1.1	NB	NB	NB	NB	NB	NB	0.7	0.6	0.7	1.1	0.8
R285A	1.5	1.1	1.2	0.8	0.9	1.0	0.9	0.8	2.6	0.9	NB	0.4
L286H	1.2	0.6	1.3?	NB	0.9	0.5	0.6	0.6	0.4	0.4	0.6	0.6
E287K	27.3	NB	NB	NB	NB	NB	NB	0.6	0.8	0.7	0.7	0.6
E291A	1.0											
R295A	11.6											
Q309A	1.0											
R312A	0.8											
K315A	0.9											
H318A	1.0	0.8	1.1	0.7	0.8	1.0	0.7	1.0	1.0	0.8	0.8	1
E320A	1.0											
E324A	1.2											
E329A	1.0											
N335A	37.8	NB	NB	0.3	NB	NB	1.5	0.6	0.5	0.5	0.5	0.8
K336A	0.9											
T338A	30.3											
H339A	1.7											
K350A	1.0											
Q358A	1.2											
K366A	0.9											
E367A	1.2											
D372A	1.2											
E373A	1.1											

【０１３０】
灰色の領域は、命名された変異体にモノクローナル抗体結合に対する初期効果がなく、それ故に再アッセイをしなかったことを示している。モノクローナル抗体の結合を完全に破壊した変異は、ＮＢ（「結合が観察されなかった」）として示されている。この分析は、試験された抗－ｔｒｋＣアゴニストモノクローナル抗体による認識における、ｔｒｋＣのアミノ酸残基Ｌ２８４，Ｅ２８７及びＮ３３５の際だった貢献を示している。ｔｒｋＣドメイン５とＮＴ３の複合体のモデルは、抗体のＣＤＲと密接に接触するこれらの残基の位置を示す（図８）。このモデルは、ｔｒｋＡドメイン５とＮＧＦの複合体の外皮構造に基づいている。さらなる詳細については、例えばＵｒｆｅｒら．Ｊ．Ｂｉｏｌ．Ｃｈｅｍ．（１９９８），上掲，又はＵｌｔｓｃｈら．，Ｊ．Ｍｏｌ．Ｂｉｏｌ．２９０：１４９－１５９（１９９９）を参照せよ。
【０１３１】
抗体可変領域のクローニング及びシークエンシング
ｔｒｋＣと抗－ｔｒｋＣモノクローナル抗体の間の相互作用の分子機序をより良く理解するために、アゴニスト抗体の重及び軽鎖可変配列をクローニングし、ＤＮＡ配列を決定した。全ＲＮＡを、Ｓｔｒａｔａｇｅｎｅ（Ｌａ Ｊｏｌｌａ，ＣＡ）のＲＮＡ単離キットを用いて、ヒト及びマウス抗－ｔｒｋＣ抗体を生産するハイブリドーマ細胞から単離した。ＳｕｐｅｒＳｃｒｉｐｔ ＩＩ システム（Ｌｉｆｅ Ｔｅｃｈｎｏｌｏｇｉｅｓ，Ｉｎｃ．，Ｇａｉｔｈｅｒｓｂｕｒｇ，ＭＤ）及び各重又は軽鎖サブグループから誘導したフレームワーク４配列に基づく特異的３’プライマーを用いて、ＲＮＡをｃＤＮＡへ逆転写した（Ｋａｂａｔ及びＷｕ，Ｊ．Ｉｍｍｕｎｏｌ．１４７：１７０９－１７１９［１９９１］）。続くＰＣＲ増幅は、重及び軽鎖のＮ－末端アミノ酸配列に基づく特異的前方向プライマー、及びｃＤＮＡ合成に用いる同じ３’プライマーを有する２．５Ｍ Ｄ

ＭＳＯの存在下でＡｍｐｌｉＴａｑ ＤＮＡポリメラーゼ（Ｐｅｒｋｉｎ Ｅｌｍｅｒ，Ｆｏｓｔｅｒ Ｃｉｔｙ，ＣＡ）を用いておこなった。ＰＣＲ産物を、ヒト重及び軽鎖定常領域の両方を含むＦ（ａｂ）’$_2$ベクターへクローニングした（Ｃａｒｔｅｒら．，Ｂｉｏ／Ｔｅｃｈｎｏｌｏｇｙ １０：１６３－１６７［１９９２］）。V_H及びV_Lドメインの各５つのクローンについて配列を決定し、コンセンサス配列を得た。

【０１３２】

図９は、抗－ｔｒｋＣアゴニストモノクローナル抗体の重鎖の推定アミノ酸配列である（２２５０，配列番号：４２；２２５３，配列番号：４３；２２５６，配列番号：４４；６．１．２，配列番号：４５；６．４．１，配列番号：４６；２３４５，配列番号：４７；及び２３４９，配列番号：４８）。抗－ｔｒｋＣアゴニストモノクローナル抗体の軽鎖の推定アミノ酸配列は、図１０に示されている（２２５０，配列番号：４９；２２５３，配列番号：５０；２２５６，配列番号：５１；６．１．２，配列番号：５３；６．４．１，配列番号：５３；２３４５，配列番号：５４；及び２３４９，配列番号：５５）。図９及び図１０の双方では、相補性決定領域（ＣＤＲ）は、ＣＤＲ１，ＣＤＲ２及びＣＤＲ３として標識され、対応するアミノ酸残基は太字で示されている。図１１は、種々の抗－ｔｒｋＣモノクローナル抗体が属する各重及び軽鎖可変ファミリーの記号表示と同様に重鎖のＣＤＲの配列を要約している。

【０１３３】

確定した抗－ｔｒｋＣアゴニストモノクローナル抗体の重及び軽鎖のＣＤＲのアミノ酸配列に基づいて、これら領域に関する幾つかの一般式を示すことは可能である。マウス抗体に関しては、重鎖ＣＤＲ１は、位置１のＸａａがＦ又はＹ、位置３のＸａａがＩ又はＭそして位置４のＸａａがＥ又はＨである、式ＸａａＷＸａａＸａａＷＶＫ（配列番号：３７）によって示すことができる。マウス重鎖ＣＤＲ２は、位置３のＸａａがＬ又はＹ、位置５のＸａａがＧ又はＳ、位置６のＸａａがＳ又はＮ、位置７のＸａａがＤ又はＧ、位置８のＸａａがＮ又はＲそして位置１７のＸａａがＧ又はＳである、式ＥＩＸａａＰＸａａＸａａＸａａＸａａＴＮＹＮＥＫＦＫＸａａ（配列番号：３８）で示すことができる。マウス重鎖ＣＤＲ３は、式ＫＮＲＮＹＹＧＮＹＶＶ（配列番号：１２）又はＫＹＹＹＧＮＳＹＲＳＷＹＦＤＶ（配列番号：１３）で示すことができる。ヒト抗体に関しては、重鎖ＣＤＲ１は、位置１のＸａａがＳ又は１（Ｌ）、位置２のＸａａがＧ又はＳそして位置３のＸａａがＧ，Ｔ又はＹ、そして位置７のＸａａがＳ又はＮである、式ＸａａＸａａＸａａＹＹＷＸａａ（配列番号：３９）によって示すことができる。ヒト重鎖ＣＤＲ２は、位置１のＸａａがＹ又はＲ、位置３のＸａａがＹ又はＦ、位置４のＸａａがＹ又はＴ、位置８のＸａａがＳ又はＲ、そして位置１０のＸａａがＮ又はＹである、式ＸａａＩＸａａＸａａＳＧＳＸａａＴＸａａＮＰＳＬＫＳ（配列番号：４０）で示すことができる。ヒト重鎖ＣＤＲ３は、ＤＲＤＹＤＳＴＧＤＹＹＳＹＹＧＭＤＶ（配列番号：１４）、ＤＧＧＹＳＮＰＦＤ（配列番号：１５）、又は位置８のＸａａがＡ又はＴ、そして位置１６のＸａａがＤ又はＡである、式ＥＲＩＡＡＡＧＸａａＤＹＹＹＮＧＬＸａａＶ（配列番号：４１）で示すことができる。

【０１３４】

重及び軽鎖可変領域の推定アミノ酸配列は、これら抗体のＮ末端ペプチド配列の決定によって確かめられる。Ｍｉｌｌｉｐｏｒｅ Ｉｍｍｏｂｉｌｏｎ－ＰＳＱ膜上へのエレクトロブロッティングは、１時間にわたって、ＢｉｏＲａｄ Ｔｒａｎｓ－Ｂｌｏｔ ｔｒａｎｓｆｅｒ ｃｅｌｌ（Ｍａｔｓｕｄａｉｒａ，Ｊ．Ｂｉｏｌ．Ｃｈｅｍ．２６２：１００３５－１００３８［１９８７］）での２５０ｍＡの定電流でおこなわれた。ＰＶＤＦ膜を、０．５分間、５０％メタノール中の０．１％ クーマシーブルーＲ－２５０で染色し、２－３分間にわたって、５０％メタノール中の１０％酢酸で脱色した。この膜を水で徹底的に洗浄し、２０℃で貯蔵する前に乾燥させた。自動化タンパク質シークエンシングを、オン－ラインＰＴＨアナライザーを装備しているモデル４９４Ａ Ｐｅｒｋｉｎ－Ｅｌｍｅｒシーケンサー（Ｐｅｒｋｉｎ－Ｅｌｍｅｒ Ｃｏｒｐｏｒａｔｉｏｎ，Ｆｏｓｔｅｒ Ｃｉｔｙ，ＣＡ）でおこなった。ＰＶＤＦ膜へエレクトロブロットしたタンパク質

を、６ｍｍのマイクロガラスカートリッジでシーケンシングした。ピークは、Ｎｅｌｓｏｎ Ａｎａｌｙｔｉｃａｌ ７６０ インターフェイスを用いたＪｕｓｔｉｃｅ Ｉｎｎｏｖａｔｉｏｎ ｓｏｆｔｗａｒｅで統合した。配列の解釈は、ＤＥＣアルファ（Ｈｅｎｚｅｌら．，Ｊ．Ｃｈｒｏｍａｔｏｇｒａｐｈｙ ４０４：４１－５２［１９８７］）でおこなった。表６は、Ｎ末端配列に基づく、ヒト及びマウス抗－ｔｒｋＣアゴニストモノクローナル抗体の分類を要約している。

【０１３５】

表６

ヒト抗-ｔｒｋＣアゴニストｍＡｂｓ	重鎖	軽鎖
６．１．２	サブグループII	カッパI
６．４．１	サブグループII	カッパI
２３４５	サブグループII	カッパIII
２３４９	サブグループII	カッパIII
２．５．１	サブグループII	カッパI
２３４４	サブグループII	カッパI
マウス抗-ｔｒｋＣアゴニストｍＡｂｓ		
２２４８	サブグループIIA	カッパI
２２５０	サブグループIIA	カッパI
２２５３	サブグループIIA	カッパIV
２２５６	サブグループIIA	カッパIII

【０１３６】

実施例２： 実験動物モデルの神経障害に対するアゴニスト抗－ｔｒｋＣモノクローナル抗体の効果

ＮＴ－３アゴニストの原則的用途は、末梢神経障害の治療及び／又は予防である。自己受容及び振動感覚の媒介に関わる、多繊維有髄化感覚ニューロンが、ＮＴ－３の高親和性レセプターとして作用するｔｒｋＣを発現することは知られている。これら多繊維に関与する神経障害は糖尿病に共通であり、さらに所定の化学療法剤、特にシスプラチン及びピリドキシンへの応答で誘導される。ＮＴ－３は、実験用糖尿病性神経障害及びシスプラチン誘導神経障害の動物モデルで有効であることが示されている。しかしながら、ＮＴ－３の用途は、げっ歯類のモデルで示されたように、その貧弱な生体利用度によって、厳しく妨害されている。ＮＴ－３のアゴニストとしての抗－ｔｒｋＣモノクローナル抗体の用途は、多くの利点を提供し、ＮＴ－３の用途に関わる多くの潜在的な課題を未然に防ぐ。

【０１３７】

アゴニスト抗－ｔｒｋモノクローナル抗体のインビボ半減期は、実験動物で静脈又は皮下のいずれかで注射することによって決定された。図１２に示されているのは、ラットでの１ｍｇ／ｋｇの静脈注射（ＩＶ）又は５ｍｇ／ｋｇの皮下注射（ｓｕｂＱ）の後の種々の時間でのモノクローナル抗体２２５６の血清レベルである。この血清のレベルは、ＫＩＲＡアッセイを用いて、ｔｒｋＣのチロシン自己リン酸化を増やすことのできる抗体２２５６の能力により完全に機能している抗体２２５６の量を測定することで決定された。これらのデータは、ラットのモノクローナル抗体２２５６が９日の半減期と、皮下投与の後は６９％の生体利用度を有していることを示す。これらの値は、他の抗体で得られた値と一致し、ＮＴ３で得られたのとは明らかに異なる。図１２に同じく示されているは、Ｍａｂ２２５６について示してあるのと同じ投与量及び経路（１ｍｇ／ｋｇ，ＩＶ；５ｍｇ／ｋｇ ｓｕｂＱ）でＮＴ－３の注射をした後に得られたデータである。これらのデータは、ＮＴ－３については、およそ４－５分の血清半減期で、７％の皮下生体利用度であることを示す。これらのデータは、生体利用度の非常に重要な特性及びインビボでの血清半減期の点から、この抗体がＮＴ－３に対して顕著な改善であることを示している。

【０１３８】

多繊維状感覚神経障害の２つの動物モデルにおいて、ＮＴ－３が化学発作の影響を防御又

は無効にできることが示されている。ＮＴ３の非常に高い投与量によって、ピリドキシンの高い投与量による毒作用から多繊維状感覚ニューロンが防御され、ＮＴ－３のさらに中程度の投与量によって、シスプラチン投与の影響が無効にされることが示されている。ＮＴ－３の組織分布及びここで記載のアゴニストＭａｂｓに多くの相違があり得ることから、Ｍａｂｓのインビトロ活性が動物モデルで効力となるどうかを確定することは重要である。

シスプラチン誘導神経障害の動物モデルを作り出すために、成人ラットへ１６週間にわたって週に２回のシスプラチンの投与を腹腔内（ＩＰ）に１ｍｇ／ｋｇでおこなった。この時点で、ラットを４つのグループへ分けた。すべての４つのグループでは、シスプラチンの週２回の投与を続けた。この継続したシスプラチン投与の他に、一つのグループでは、ＮＴ－３の１ｍｇ／ｋｇの投与を週に３回受け、一つのグループでは、週に１回のＭａｂ２２５６の１ｍｇ／ｋｇの投与を受け、一つのグループでは、週に１回のＭａｂ６．４．１の１ｍｇ／ｋｇの投与を受け、そして一つのグループでは、週に３回の生理的食塩水の投与を受けた。Ｍａｂ及び生理的食塩水をＩＶで投与されたのに対して、ＮＴ－３投与は皮下で投与された。この治療方法は、さらに４週間にわたって続けられ、合計で２０週間のシスプラチン投与であった。

【０１３９】

多繊維状感覚ニューロンの機能を、Ｈ－波を記録することで、これら動物を電気生理学的に評価した（Ｇａｏら．，Ａｎｎ．Ｎｅｕｒｏｌ．３８（１）：３０－７［１９９５］）。図１３に示してあるデータに見られるように、生理的食塩水のみとシスプラチンで処理した動物では、感覚伝導速度は非常に低かった。週に３回のＮＴ－３処理によって、Ｍａｂ２２５６又はＭａｂ６．４．１のいずれかによる週１回の治療のように、この低下した電動速度の向上が生じた。週に１回のモノクローナル抗体による向上の規模は、少なくともＮＴ－３で週に３回治療することで見られるのと同じ規模である。

ピリドキシンは、感覚ニューロンの多有髄化した亜集団を主に傷つける感覚神経障害を誘導することでも知られている（Ｈｅｌｇｒｅｎら．，Ｊ．Ｎｅｕｒｏｓｃｉ．１７（１）：３７２－８２［１９９７］）。ＮＴ－３の大量投与によって、この神経障害の進行が阻止されることが知られている（Ｈｅｌｇｒｅｎら．，上掲）。２週間にわたる、ピリドキシンの２つの異なる投与（１日に４００ｍｇ／ｋｇ又は６００ｍｇ／ｋｇで腹腔内投与）での動物の治療によって、ＤＲＧの多ニューロンに傷害が生じる。この傷害は、ＤＲＧの多ニューロンによって優先的に又は独占的にのいずれかで発現することが知られている幾つかのタンパク質の減少によって検出が可能である。これらマーカーの発現レベルは、ＴＡＱＭＡＮ ＲＴ－ＰＣＲ技術を用いることによって、それらをコードしているｍＲＮＡのレベルを測定することで評価される。

【０１４０】

ｔｒｋＣアゴニスト効果のためのTaqman RT-PCR

Ａ．プローブ及びプライマー

ＮＦＬ

F-CAGCAGAACAAGGTCCTGGAA　21MER（配列番号：７２）
R-AGCGGGAAGGCTCTGAGTG　19MER　（配列番号：７３）
P-AGCTGTTGGTGCTGCGCCAGAA　22MER　（配列番号：７４）

ＮＳＥ

F-TCCATTGAAGACCCATTCGAC　21MER（配列番号：７５）
R-GCCGACATTGGCTGTGAAC　19MER（配列番号：７６）
P-AGGATGACTGGGCAGCTTGGTCCA　24MER（配列番号：７７）

ＴＲＫＣ

F-CAGCCCACTGCACCATATCA　20MER（配列番号：７８）
R-CTGTATCCGGCCCAGCAT　18MER（配列番号：７９）
P-CCATGGCATCACTACACCTTCATCGCT　27MER（配列番号：８０）

ＣＡＬＲＥＴ

F-TGGGAAAATTGAGATGGCAGA　21MER（配列番号：８１）
R-GCTGCCTGAAGCACAAAAGG　20MER（配列番号：８２）
P-CGCAGATCCTGCCAACCGAAGAGA　26MER（配列番号：８３）

ＰＡＲＶＡＬＢ

F-GACACCACTCTTCTGGAAAATGC　23MER（配列番号：８４）
R-TTGCCAAACCAACACCTACCA　21MER（配列番号：８５）
P-ATCGGACACCACCTGTAGGGAGGACC　26MER（配列番号：８６）

ＧＡＰＤＨ

F-CAGTGGCAAAGTGGAGATTGT　21MER（配列番号：８７）

R-AATTTGCCGTGAGTGGAGTC　20MER（配列番号：８８）
P-CCATCAACGACCCCTTCATTGACCTC　26MER（配列番号：８９）

プローブ及びプライマーを、Ｐｒｉｍｅｒ Ｅｘｐｒｅｓｓ（ＡＢＩ－Ｐｅｒｋｉｎ－Ｅｌｍｅｒ）を用いて設計した。プライマープローブの選択に関するガイドラインは、Ｗｉｌｌｉａｍｓ ａｎｄ Ｔｕｃｋｅｒ（１９９９）ＰＣＲ ａｐｐｌｉｃａｔｉｏｎｓ，ｐｐ．３６５－７５（Ａｃａｄｅｍｉｃ Ｐｒｅｓｓ）に含まれている。

【０１４１】

Ｂ．全ＲＮＡの調製及び定量化

Ｌ４及びＬ５を、リン酸バッファー化生理的食塩水を灌流させたラットから解剖して取り出した。左及び右側を別々の試験管に単離した。標準曲線で用いた全ＲＮＡのために、すべてのＤＲＧをコントロールのラットから解剖して取り出した。全ＲＮＡを、Ｑｉａｇｅｎ Ｒｎｅａｓｙ ｍｉｎｉ ｃｏｌｕｍｎｓを用いて単離した。組織を、製造者指示書によってホモジナイズした。全ＲＮＡは、Ｒｉｂｏｇｒｅｅｎ Ｑｕａｎｔｉｔａｔｉｏｎ Ｋｉｔ（Ｍｏｌｅｃｕｌａｒ Ｐｒｏｂｅｓ）を用いて、製造者指示書に従って定量化した。

【０１４２】

Ｃ．ＲＴ－ＰＣＲ

反応当たり５００，２５０，２５又は２．５ナノグラムを使用した標準曲線を除いて、全ＲＮＡの２５ナノグラムを５０μｌ反応に用いた。各反応は、２５ｐｍｏｌの各オリグヌクレオチドプライマー、０．２ｍＭの各ｄＮＴＰ、１００ｎＭの蛍光標識オリゴヌクレオチドプローブ、１Ｘ ＲＴ－ＰＣＲバッファー（ＰＥ ｂｉｏｓｙｓｔｅｍｓ）、２．０ｍ

ＭＭｇＣｌ２、２０Ｕ ＲＮＡｓｅインヒビター、１２．５ＭｕＬＶ逆転写酵素（ＲＴ，ＰＥ ｂｉｏｓｙｓｔｅｍｓ）及び２．５Ｕ Ａｍｐｌｉｔａｑ Ｇｏｌｄ ｐｏｌｙｍｅｒａｓｅ（ＰＥ ｂｉｏｓｙｓｔｅｍｓ）を含む。逆転写は、４８℃で３０分にわたっておこない、続いて９５℃で１０分間のＡｍｐｌｉｔａｑ Ｇｏｌｄ 活性化及びＲＴ不活性化、次いでＰＣＲ；１５秒にわたる９５℃及び１．５分にわたる６０℃の４０サイクルでおこなった。

【０１４３】

Ｄ．遺伝子発現の定量化

コントロールＲＮＡを用いて、ハウスキーピング遺伝子及び対象遺伝子の各ｔａｑｍａｎの実行に関する標準曲線を作成した。標準曲線を添加したコントロールの全ＲＮＡ量の対数に対するＴａｑｍａｎの実行より得られた閾値（Ｃｔ）をプロットすることで得られた。ＲＮＡ対数値に関して、結果として得られた一次方程式を解いた。実験から得られたＣｔ値を埋め合わせることで、実験から得られる遺伝子発現値の対数が得られた。この値の１０乗は、実験上の遺伝子発現をナノグラムで示す。

図１４に見られるように、２週間にわたるピリドキシン治療によって、神経細線維軽鎖（ＮＦＬ）、ニューロン特異的エノラーゼ（ＮＳＥ）、ｔｒｋＣ、及びカルレチニン発現の用量依存の減少を引き起こす。用量依存及びこれら減少の大きさの双方は、マーカーの間で異なり、神経細胞損傷のマーカーとしてこれらタンパク質の差次的感度を示す。

【０１４４】

図１５では、ピリドキシンの低用量（毎日４００ｍｇ／ｋｇ）に加えて、Ｍａｂ２２５６の２倍用量で動物を治療した結果を示している。ＮＦＬ及びＮＳＥは、このレベルのピリドキシン治療での発現の顕著な減少を示す。５ｍｇ／ｋｇのＭａｂ２２５６（毎週ｓｕｂＱ）で動物を同時治療することで、発現におけるこの減少が完全に阻止された。１ｍｇ／ｋｇのＭａｂ２２５６の服用は、これらタンパク質の発現に対して明かな効果を有しなかった。ｔｒｋＣ又はカルレチニン発現のいずれも、この低用量のピリドキシン治療によって顕著な影響を受けず、５ｍｇ／ｋｇのＭａｂ２２５６による治療によって、ｔｒｋＣの発現が実際にコントロールのレベルを超える。

毎日６００ｍｇ／ｋｇのより高いピリドキシン用量で動物を治療した場合、ｔｒｋＣの発現がコントロール値のおよそ５０％に落ちる一方で、ＮＦＬ、ＮＳＥ及びカルレチニンの発現が非常に低いレベルに落ちる（図１６）。１ｍｇ／ｋｇ又は５ｍｇ／ｋｇのいずれかのＭａｂ２２５６による同時治療は、完璧にではないが顕著にｔｒｋＣ及びカルレチニンに見られる発現の減少を阻止する。Ｍａｂ２２５６で治療した動物でのＮＦＬ及びＮＳＥ発現で見られる防御への僅かな傾向があるが、それは統計的に顕著な値までには達しなかった。従って、多感覚ニューロンに対する損傷の複数の生化学的マーカーを用いると、Ｍａｂ２２５６がピリドキシン治療の毒性を改善することができると思われる。

【０１４５】

ピリドキシン神経障害の電気生理学的及び行動への効果を検討するために、ラットを８日間、１日に２回の４００ｍｇ／ｋｇピリドキシンの注射で治療した。それらの多直径感覚求心性神経を、太腿及びふくらはぎの座骨神経の刺激後の足の筋肉におけるＭ波（直接運動）及びＨ波（反射性感覚）反応を記録することによって、電気生理学的に試験した（Ｇａｏら．，Ａｎｎ．Ｎｅｕｒｏｌ．３８（１）：３０－７［１９９５］）。８日間のピリドキシンによる治療は、図１８に見られるような運動応答に比べて、感覚応答の振幅にかなりの減少を引き起こした。Ｍａｂ２２５６による共治療は、感覚振幅でのピリドキシン誘導減少を顕著に阻止した。これは、非常に高い用量（毎日２０ｍｇ／ｋｇ）のＮＴ－３を用いた公開された効果に類似している（Ｈｅｌｇｒｅｎら．，上掲）。

【０１４６】

ピリドキシンのこの療法で治療した動物は、また、固有受容性機能に関しても行動上の点から試験された。動物は、明るい灯と白色雑音の刺激を避けるために、雲梯を歩いてわたって暗い箱へ行くように訓練された。動物は、下からビデオテープで録画され、はしごの段上の後ろの足の配置の特徴は、それらの治療が分からない観察者によって判断された。

各足の配置は、良い配置（足が、つま先の直ぐ後ろで、中足の前部分に着き、つま先ははしごの段を直接に巻きつく）、しっかりとした着地（足は、直ぐ後ろのつま先以外に当たるが、はしごの段にしっかりと着き、つま先は殆どの場合は巻き付かない）、ほぼフットフォールト（つま先の極端に前部分又はかかとの後部のいずれかで、足がはしごの段にかろうじて当たるが、体重を支えない）、又はフットフォールト（足が、はしごの段を完全にはずしているか、又は非常に弱い配置であって、足は体重を支えず、体重支持面へはしごを通して落下する）のように記録された。正常なラットは、非常に早く後ろ足を正確におくことを習得し、それは、スペースでの後ろ足の位置についての優れた固有受容性の感覚を必要とする。ピリドキシンによる治療の後（８日間、毎日４００ｍｇ／ｋｇを２回）、この課題に関する成績は低下し、良好な配置についてはおよそ３０パーセントの減少があり、フットフォールト及びほぼフットフォールトの双方で増加があった（図１８）。この回の間のＭａｂ２２５６による動物の同時治療では、良好な配置におけるより小さな減少、そしてフットフォールト及びほぼフットフォールトでのより小さな増加をともなう、より高い程度の成績を動物が維持することを可能にした。

【０１４７】

要約すると、Ｍａｂ２２５６による共治療は、生化学的、電気生理学的、そして行動課題についての成績によって測定されるピリドキシンの毒性効果を改善する。

ｔｒｋＣ Ｍａｂが、治療的には少なくともＮＴ－３と同じ程度に効果的あることが立証された後、観察された痛覚過敏の逆現象が検証された。ＮＴ－３投与のこの副作用は、げっ歯（図１９を参照せよ）及びヒト（Ｃｈａｕｄｈａｒｙら．，Ｍｕｓｃｌｅ ａｎｄ Ｎｅｒｖｅ ２３：１８９－１９２［２０００］）で見出されている。ラットを、ハーグリーブス装置を用いた後ろ足の温度感受性のために訓練して試験し、次いで１ｍｇ／ｋｇのＭａｂ２２５６ＩＶ、又は１ｍｇ／ｋｇのＮＴ－３を首筋の皮下に投与した。投与の後の２，４，６時間、ラットを、再び熱離脱時間について試験した。図１９からわかるように、ＮＴ－３投与は、投与後の４及び６時間で顕著な心臓痛覚過敏を起こし、一方でｔｒｋＣ Ｍａｂ２２５６は、熱痛み感覚への効果はなかった。従って、神経障害を逆進又は防ぐのに効果的であることが知られている服用量では、ＮＴ－３は、痛みに対する感受性を増し、一方でＭａｂ２２５６は増さない。

【０１４８】

広く用いられている化学療法剤であるシスプラチンは、振動感覚及び固有受容の選択的損失をともなう感覚神経障害を誘導する。ここで、我々は、神経栄養因子の神経成長因子ファミリーのメンバーであるニューロトロフィン－３（ＮＴ－３）が正常なレベルである、ラットのシスプラチンによって誘導された減少Ｈ－反射関連感覚神経伝導速度の状態へ回復したことを示す。ＮＴ－３治療は、背根神経節の多感覚ニューロンでの神経細線維タンパク質の異常な細胞室内分布、及びシスプラチンによって生じた腓腹神経の中の有髄繊維の数の減少を正す。従って、シスプラチン神経毒性のＮＴ－３依存性反転は、末梢感覚神経障害の治療でのＮＴ－３の用途の可能性を示唆する。

【０１４９】

２－３週間にわたる、ピリドキシン（ビタミンＢ６）の高服用量による成人ラットの長期にわたる治療によって、このビタミンの過剰服用又は化学療法剤シスプラチンで治療したヒトで見出されるのと類似した深刻な固有受容の損失を生じた。ピリドキシンの毒性は、単純で正確な移動運動及び感覚神経機能が欠失、多直径／多繊維脊髄感覚ニューロンの変性として明かである。ビームウォーキングタスクで、そして末梢神経刺激によって惹起されたＨ波のＥＭＧ記録によって定量的に見積もられたように、長期にわたるピリドキシン治療の間の神経栄養因子ニューロトロフィン－３（ＮＴ－３；５－２０ｍｇ／ｋｇ／日、皮下注射）の同時投与は、ピリドキシン毒性に関連する行動性及び電気生理学的な後遺症をかなり和らげる。さらに、ＮＴ－３投与は、脊髄の脊柱の中の感覚繊維の変性を防ぐ。これらのデータは、ＮＴ－３が筋肉感覚求心性神経に対する標的誘導神経栄養因子であり、ＮＴ－３の薬学的服用量が、多繊維感覚神経障害の治療にとって有益であり得ることを示唆している。

【０１５０】
生物学的材料の寄託
次のハイブリドーマ細胞株及びプラスミドをアメリカン・タイプ・カルチャー・コレクション，１０８０１ ユニバーシティー ブルーバード， マナサス， バージニア， ２０１１０－２２０９ アメリカ合衆国（ＡＴＣＣ）へ２０００年６月２１日寄託した：

ハイブリドーマ／プラスミド命名	ＡＴＣＣＮｏ．
２．５．１	ＰＴＡ－２１５１
６．１．２	ＰＴＡ－２１４８
６．４．１	ＰＴＡ－２１５０
２３４４	ＰＴＡ－２１４４
２３４５	ＰＴＡ－２１４６
２３４９	ＰＴＡ－２１５３
２２４８	ＰＴＡ－２１４７
２２５０	ＰＴＡ－２１４９
２２５３	ＰＴＡ－２１４５
２２５６	ＰＴＡ－２１５２
ＤＮＡ ｐＸＣＡ－２２５０ＨＬ	ＰＴＡ－２１３６
ＤＮＡ ｐＸＣＡ－２２５３ＨＬ	ＰＴＡ－２１３７
ＤＮＡ ｐＸＣＡ－２２５６ＨＬ	ＰＴＡ－２１３８
ＤＮＡ ｐＸＣＡ－６．１．２Ｈ	ＰＴＡ－２１４１
ＤＮＡ ｐＸＣＡ－６．４．１Ｈ	ＰＴＡ－２１４３
ＤＮＡ ｐＸＣＡ－２３４５Ｈ	ＰＴＡ－２１４２
ＤＮＡ ｐＸＣＡ－２３４９Ｈ	ＰＴＡ－２１３３
ＤＮＡ ｖｅｇｆ４ｃｈｉｍ－６．１．２Ｌ	ＰＴＡ－２１３４
ＤＮＡ ｖｅｇｆ４ｃｈｉｍ－６．４．１Ｌ	ＰＴＡ－２１３５
ＤＮＡ ｖｅｇｆ４ｃｈｉｍ－２３４５Ｌ	ＰＴＡ－２１３９
ＤＮＡ ｖｅｇｆ４ｃｈｉｍ－２３４９Ｌ	ＰＴＡ－２１４０

【０１５１】
これらの寄託は、特許手続き上の微生物の寄託の国際的承認に関するブダペスト条約及びその規則（ブダペスト条約）の規定に従って行われた。これは、寄託の日付から３０年間、寄託の生存培養物が維持されることを保証するものである。寄託物はブダペスト条約の条項に従い、またジェネンテク社とＡＴＣＣとの間の合意に従い、ＡＴＣＣから入手することができ、これは、何れが最初に来ようとも、関連した米国特許の発行時又は任意の米国又は外国特許出願の公開時に、寄託培養物の後代を永久かつ非制限的に入手可能とすることを保証し、米国特許法第１２２条及びそれに従う特許庁長官規則（特に参照番号８８６ＯＧ６３８の３７ＣＦＲ第１．１２条を含む）に従って権利を有すると米国特許庁長官が決定した者に子孫を入手可能とすることを保証するものである。
【０１５２】
欧州特許を求めるこれら記号表示に関する限りでは、寄託微生物の試料は、その試料を請求している人によって指名された専門家に対するそのような試料の発行のみによって、欧州特許付与の言及についての公開まで、又は出願が拒絶又は取り下げられた、又は取り下げられると思われる日まで入手可能である（Ｒｕｌｅ ２８（４）ＥＰＣ）。
本出願の譲受人は、寄託した材料の培養物が、適切な条件下で培養されていた場合に死亡もしくは損失又は破壊されたならば、材料は通知時に同一の他のものと速やかに取り替えることに同意する。寄託物質の入手可能性は、特許法に従いあらゆる政府の権限下で認められた権利に違反して、本発明を実施するライセンスであるとみなされるものではない。
【０１５３】
上記の文書による明細書は、当業者に本発明を実施できるようにするために十分であると考えられる。寄託した態様は、本発明のある側面の一つの説明として意図されており、機能的に等価なあらゆる作成物がこの発明の範囲内にあるため、寄託された作成物により、

本発明の範囲が限定されるものではない。ここでの材料の寄託は、ここに含まれる文書による説明が、そのベストモードを含む、本発明の任意の側面の実施を可能にするために不十分であることを認めるものではないし、それが表す特定の例証に対して請求の範囲を制限するものと解釈されるものでもない。実際、ここに示し記載したものに加えて、本発明を様々に変形することは、前記の記載から当業者にとっては明らかなものであり、添付の請求の範囲内に入るものである。

【０１５４】

本発明の教示の特定の課題又は状況へ応用することが、ここに包含されている教示の観点から、当該分野の通常の技術を有する者の能力の範囲内にあることは理解されている。本発明の産物の例，及びそれらの単離、用途，及び製造に関する代表的な工程が下に示されるが、本発明を限定するよう構成されるものではない。

本明細書中のすべての引用文献及びそれに引用されている引用文献は、出典明示によってここに明示的に取り込む。

【図面の簡単な説明】

【図１Ａ－Ｄ】ＫＩＲＡ（Ａ及びＢ）及びＰＣ１２神経突起増殖アッセイ（Ｃ及びＤ）を用いた例示されたｔｒｋＣレセプターに対する種々のヒト（Ａ及びＣ）及びマウス（Ｂ及びＤ）モノクローナル抗体のアゴニスト活性を示す。プロテインＡ精製モノクローナル抗体を、ＫＩＲＡ刺激バッファー（２％ウシ血清アルブミン［ＢＳＡ，Ｉｎｔｅｒｇｅｎ Ｃｏ．，　Ｐｕｒｃｈａｓｅ，ＮＹ］及び２５ｍＭ　Ｈｅｐｅｓを含む、０．２μｍの濾過されたＦ１２／ＤＭＥＭ　５０：５０）で２７μｇ／ｍｌに希釈した。次いで、モノクローナル抗体を、刺激培地で１：３（全体で８希釈；濃度範囲は０．０１－１８０ｎＭ　Ｎａｂ）に希釈した。次いで、ＧＤ－トランスフェクトＣＨＯ細胞（5×10^4 細胞／ウェル）を６時間にわたってＮＴ－３又はＭａｂ（二重にアッセイした希釈液）のいずれかで刺激し、このアッセイを実施例に記載のように完結させた（Ｆｉｇ．１Ａ、ヒト Ｍａｂｓ；Ｆｉｇ．１Ｂ、マウスＭａｂｓ）。この精製Ｍａｂｓを実施例に記載されているような、ＰＣ１２神経突起増殖アッセイでアゴニスト活性についてアッセイした。ラットＰＣ１２細胞を完全長ヒトｔｒｋＣでトランスフェクトし、この細胞を１０００細胞／ウェルの密度でプレートした。トランスフェクションの３日後、Ｍａｂｓを、ｔｒｋＣトランスフェクタントを含むウェルへ三通り（０．０００２から２．７ｎＭの濃度範囲）で加え、３７℃で更なる３日間にわたってインキュベートした。次いで、この細胞を位相差顕微鏡法によって分析し、細胞の直径の２倍を超える神経突起を有する細胞の数を数えた。

【図２】６．１．２抗体を代表的例として用いて、アゴニスト抗－ｔｒｋＣモノクローナル抗体がｔｒｋＣと特異的に結合することを示す。

【図３】アゴニスト抗－ｔｒｋＣモノクローナル抗体が、ラットｔｒｋＣよりも効率的にヒトｔｒｋＣを認識することを例示する。モノクローナル抗体のラットｔｒｋＣと結合する能力は、レセプターの免疫アドヘシン構築物を用いて決定される。ＴｒｋＣ（ヒトｔｒｋＣ－ｇＤ又はラットｔｒｋＣ－ＩｇＧ）を、終夜にわたってマイクロタイタープレート（５０ｍＭ 炭酸塩バッファー、ｐＨ９．５で１００μｌの１μｇ／ｍｌ溶液に希釈）上に固定化した。このプレートを洗浄してブロックした。次いで、Ｍａｂｓを０．５％ＢＳＡ及び０．０５％Ｔｗｅｅｎ２０を含むＰＢＳで１μｇ／ｍｌに希釈し、適切なウェル（１００μｌ／ウェル）へ添加し、そして室温で１時間にわたってインキュベートした。このプレートを洗浄し、適切なＨＲＰコンジュゲートを添加し（ヒトＭａｂｓ：ヤギ抗－ヒトκ－ＨＲＰ、１：５Ｋ；マウス Ｍａｂｓ：ヤギ抗－ｍｏｌｇＧ（Ｆｃ）－ＨＲＰ、１：５Ｋ）、そして室温で１時間にわたってインキュベートした。次いで、このプレートを洗浄し、展開して読みとった。

【図４】競合ＥＬＩＳＡを用いたエピトープマッピングの代表的な例を示す。過度の種々の非標識抗－ｔｒｋＣモノクローナル抗体の非存在又は存在下で、ビオチン化ヒト抗－ｔｒｋＣ６．１．２モノクローナル抗体を固定化ｔｒｋＣでインキュベートした。

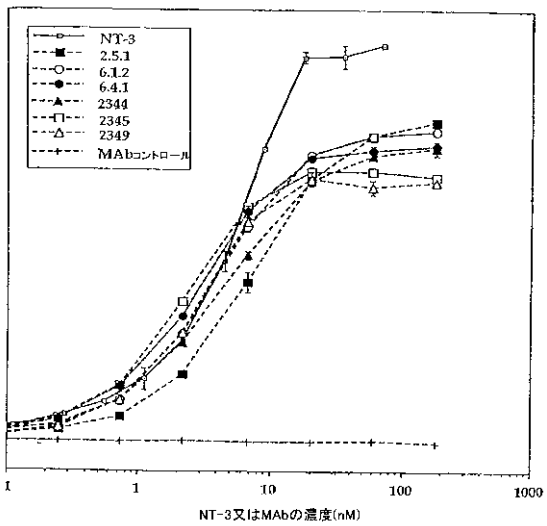
【図５】競合ＥＬＩＳＡを用いたエピトープマッピングの結果を要約する。

【図６Ａ－Ｃ】種々のｔｒｋＣキメラ（Ａ）の説明図、及び種々のアゴニストヒト（Ｂ）

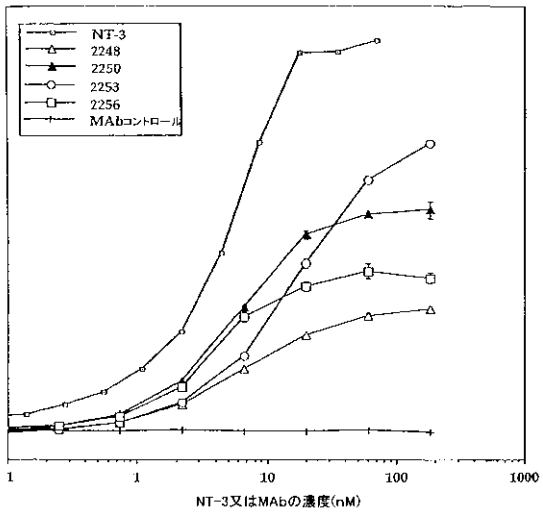
及びマウス（Ｃ）抗－ｔｒｋＣモノクローナル抗体によって認識されるｔｒｋＣ上のエピトープのマッピングへのそれらの用途を示す。
【図７】アゴニスト抗－ｔｒｋＣモノクローナル抗体による認識によってそれらの役割を解読するための突然変異誘発の標的であった残基を示している、ヒトｔｒｋＣドメイン４及び５のアミノ酸配列を示す。
【図８】抗－ｔｒｋＣモノクローナル抗体との複合体であるｔｒｋＣの３次元リボン・ダイヤグラムを示す。特に示されているのは、抗－ｔｒｋＣ抗体のＣＤＲｓによる認識において重要な役割を担う可能性があるｔｒｋＣのアミノ酸残基。
【図９】マウス及びヒト抗－ｔｒｋＣアゴニストモノクローナル抗体の重鎖可変（V_H）領域のアミノ酸配列。さらに、３つのＣＤＲ領域（ＣＤＲ１、ＣＤＲ２及びＣＤＲ３）が太字で強調されている。２２５０及び２２５３重鎖のＣＤＲ１のアミノ酸配列は、配列番号：１である。２２５６重鎖のＣＤＲ１のアミノ酸配列は、配列番号：２である。６．１．２及び２３４５重鎖のＣＤＲ１のアミノ酸配列は、配列番号：３である。６．４．１重鎖のＣＤＲ１のアミノ酸配列は、配列番号：４である。２３４９重鎖のＣＤＲ１のアミノ酸配列は、配列番号：５である。２２５０及び２２５３重鎖のＣＤＲ２のアミノ酸配列は、配列番号：６である。２２５６重鎖のＣＤＲ２のアミノ酸配列は、配列番号：７である。６．１．２重鎖のＣＤＲ２のアミノ酸配列は、配列番号：８である。６．４．１重鎖のＣＤＲ２のアミノ酸配列は、配列番号：９である。２３４５重鎖のＣＤＲ２のアミノ酸配列は、配列番号：１０である。２３４９重鎖のＣＤＲ２のアミノ酸配列は、配列番号：１１である。２２５０及び２２５３重鎖のＣＤＲ３のアミノ酸配列は、配列番号：１２である。２２５６重鎖のＣＤＲ３のアミノ酸配列は、配列番号：１３である。６．１．２重鎖のＣＤＲ３のアミノ酸配列は、配列番号：１４である。６．４．１重鎖のＣＤＲ３のアミノ酸配列は、配列番号：１５である。２３４５重鎖のＣＤＲ３のアミノ酸配列は、配列番号：１６である。２３４９重鎖のＣＤＲ３のアミノ酸配列は、配列番号：１７である。
【図１０】マウス及びヒト抗－ｔｒｋＣアゴニストモノクローナル抗体の軽鎖可変（V_L）領域のアミノ酸配列を示す。さらに、３つのＣＤＲ領域（ＣＤＲ１、ＣＤＲ２及びＣＤＲ３）が太字で強調されている。２２５０軽鎖のＣＤＲ１のアミノ酸配列は、配列番号：１８である。２２５３軽鎖のＣＤＲ１のアミノ酸配列は、配列番号：１９である。２３５６軽鎖のＣＤＲ１のアミノ酸配列は、配列番号：２０である。６．１．２軽鎖のＣＤＲ１のアミノ酸配列は、配列番号：２１である。６．４．１軽鎖のＣＤＲ１のアミノ酸配列は、配列番号：２２である。２３４５軽鎖のＣＤＲ１のアミノ酸配列は、配列番号：２３である。２３４９軽鎖のＣＤＲ１のアミノ酸配列は、配列番号：２４である。２２５０軽鎖のＣＤＲ２のアミノ酸配列は、配列番号：２５である。２２５３軽鎖のＣＤＲ２のアミノ酸配列は、配列番号：２６である。２２５６重鎖のＣＤＲ２のアミノ酸配列は、配列番号：２７である。６．１．２軽鎖のＣＤＲ２のアミノ酸配列は、配列番号：２８である。６．４．１軽鎖のＣＤＲ２のアミノ酸配列は、配列番号：２９である。２３４５及び２３４９軽鎖のＣＤＲ２のアミノ酸配列は、配列番号：３０である。２２５０軽鎖のＣＤＲ３のアミノ酸配列は、配列番号：３１である。２２５３軽鎖のＣＤＲ３のアミノ酸配列は、配列番号：３２である。２２５６軽鎖のＣＤＲ３のアミノ酸配列は、配列番号：３３である。６．１．２軽鎖のＣＤＲ３のアミノ酸配列は、配列番号：３４である。６．４．１軽鎖のＣＤＲ３のアミノ酸配列は、配列番号：３５である。２３４５及び２３４９軽鎖のＣＤＲ３のアミノ酸配列は、配列番号：３６である。
【図１１】マウス及びヒト抗－ｔｒｋＣアゴニストモノクローナル抗体の重及び軽可変鎖のＣＤＲのアミノ酸配列を示す。さらに示されているのは、データベースから入手可能なＣＤＲ配列との相同体に基づいた、これら配列のファミリーである。
【図１２】抗－ｔｒｋＣアゴニストモノクローナル抗体は、インビボでの半減期及び生体利用度が向上したことを示す。
【図１３】シスプラチン誘導神経障害に対する抗－ｔｒｋＣアゴニストモノクローナル抗体の効果を示す。
【図１４】ピリドキシン神経障害によって生じたマーカー発現の減少を示す。

【図１５】アゴニスト抗－ｔｒｋＣモノクローナル抗体による、ピリドキシンの低投与量での効果の向上を示す。
【図１６】アゴニスト抗－ｔｒｋＣモノクローナル抗体による、ピリドキシンの高投与量の効果の改善を示す。
【図１７】抗－ｔｒｋＣアゴニストモノクローナル抗体による、ピリドキシン神経障害の改善を示す。
【図１８】アゴニスト抗－ｔｒｋＣ抗体による、ピリドキシン誘導によるはしご状の欠損の減衰を示す。
【図１９】抗－ｔｒｋＣアゴニストモノクローナル抗体ではなく、ＮＴ３が治療的投与量で痛覚過敏を起こすことを示す。
【図２０】ドメイン４及び５の境界が示されている、ヒトｔｒｋＣレセプター（配列番号：５６）のアミノ酸配列を示す。
【図２１Ａ－Ｂ】ヒトｔｒｋＣレセプターのヌクレオチド配列（配列番号：５７）を示す（２項）。
【図２２】抗－ｔｒｋＣアゴニストモノクローナル抗体２２５０の重鎖（Ａ；配列番号：５８）及び軽鎖（Ｂ；配列番号：５９）のヌクレオチド配列を示す。
【図２３】抗－ｔｒｋＣアゴニストモノクローナル抗体２２５３の重鎖（Ａ；配列番号：６０）及び軽鎖（Ｂ；配列番号：６１）のヌクレオチド配列を示す。
【図２４】抗－ｔｒｋＣアゴニストモノクローナル抗体２２５６の重鎖（Ａ；配列番号：６２）及び軽鎖（Ｂ；配列番号：６３）のヌクレオチド配列を示す。
【図２５】抗－ｔｒｋＣアゴニストモノクローナル抗体２３４５の重鎖（Ａ；配列番号：６４）及び軽鎖（Ｂ；配列番号：６５）のヌクレオチド配列を示す。
【図２６】抗－ｔｒｋＣアゴニストモノクローナル抗体２３４９の重鎖（Ａ；配列番号：６６）及び軽鎖（Ｂ；配列番号：６７）のヌクレオチド配列を示す。
【図２７】抗－ｔｒｋＣアゴニストモノクローナル抗体６．１．２の重鎖（Ａ；配列番号：６８）及び軽鎖（Ｂ；配列番号：６９）のヌクレオチド配列を示す。
【図２８】抗－ｔｒｋＣアゴニストモノクローナル抗体６．４．１の重鎖（Ａ；配列番号：７０）及び軽鎖（Ｂ；配列番号：７１）のヌクレオチド配列を示す。

【図１Ａ】



【図１Ｂ】



【図１Ｃ】

表3

Mab ID	アイソタイプ	アゴニスト活性 KIRA/PC12	ラット trkC との結合	イムノブロット NR/Red. NR Red.	親和性 Kd(nM)
ヒト Mabs					
2.5.1	G2	(+++/+++)	NO	++ ++	12
6.1.2	G2	(++++/++++)	NO	+ +	12.5
6.4.1	G2	(++++/++++)	YES	+ +	12
2344	G2	(+++/+++)	NO	++ +	19
2345	G2	(++++/++++)	NO	++ ++	12.1
2349	G4	(++++/++++)	NO	++ +	23
マウス Mabs					
2248	G2a	(+/+)	NO	+++ -	5.9
2250	G1	(++/++)	NO	++ ++	8.7
2253	G1	(++/+)	NO	++ ++	42
2256	G1	(+/+)	YES	++ +	62

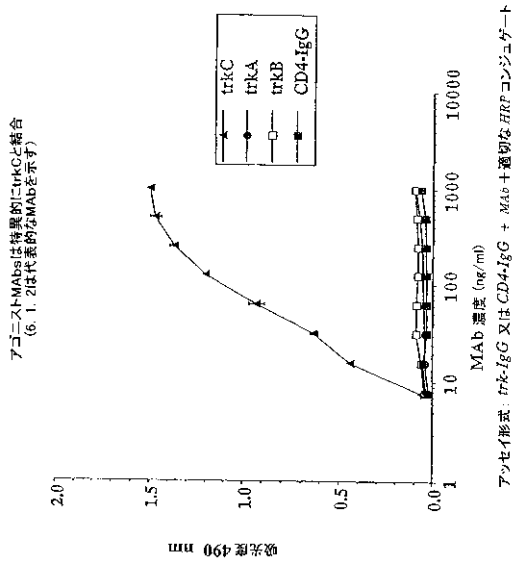
【図１Ｄ】

表4

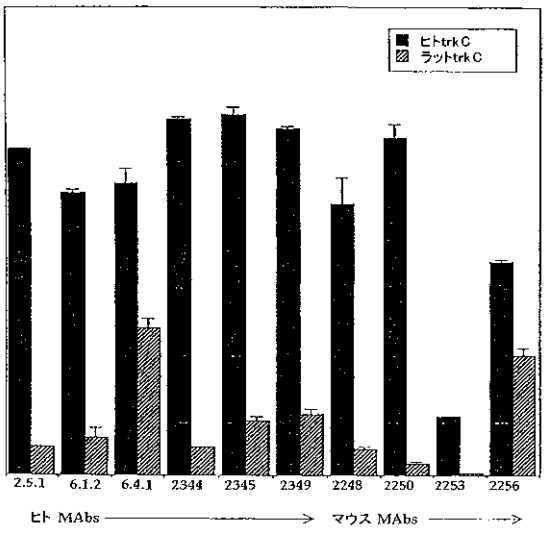
ＳＰＲによって測定した結合親和性。固定化ｇＤ-ｔｒｋＣ（４００-６００ ＲＵ）へのｉｇＧの結合、及び固定化ｉｇＧ（１０００-３０００ ＲＵ）へのｇＤ-ｔｒｋＣの結合に関する結果を示す。ＮＤＢ＝検出できない結合。

	Kd(nM)	
抗体（IgG）	固定化 gD-trkC	固定化 IgG
2248	5.9	8.5
2250	8.7	28
2253	42	51
2256	62	300
2344	19	NDB
2345	12	NDB
2349	23	NDB
6.4.1	12	28
6.1.2	13	16
2.5.1	12	NDB

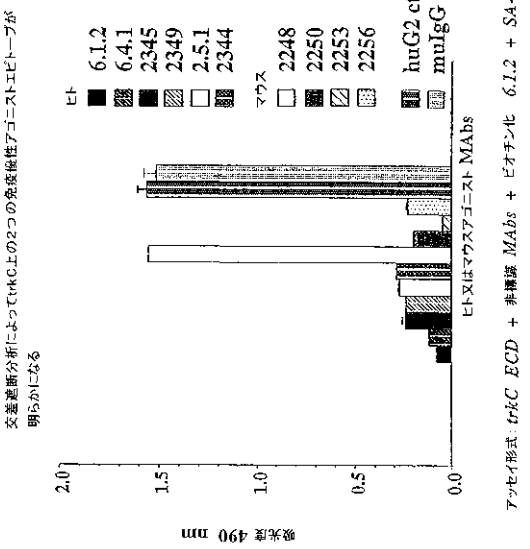
【図２】



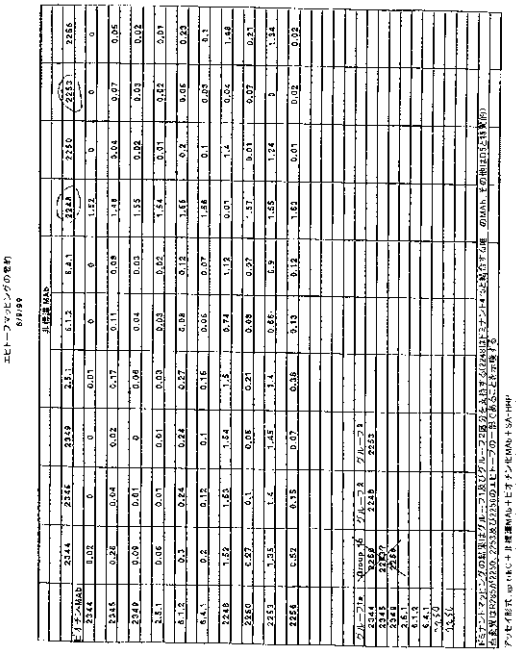
【図３】



【図４】



【図５】



エピトープマッピングの要約

	2344	2346	2349	2.5.1	6.1.2	6.4.1	2345	2250	2253	2256
ビオチン-MAb	2344	6.02	0	0	0.01	0	0	1.82	0	0
	2346	0.28	0.04	0.02	0.17	0.11	0.08	1.48	5.04	0.07	0.95
	2349	0.09	0.51	0	0.04	0.04	0.03	1.55	0.02	0.03	0.02
	2.5.1	0.06	0.01	0.01	0.03	0.03	0.05	1.54	0.01	0.02	0.01
	6.1.2	0.2	0.24	0.24	0.52	0.09	0.12	1.61	0.2	0.05	0.03
	6.4.1	0.2	0.12	0.1	0.16	0.05	0.07	1.66	0.1	0.03	0.1
	2248	1.82	1.65	1.84	1.5	0.74	1.12	0.01	1.4	0.04	1.48
	2250	0.07	0.1	0.05	0.21	0.03	0.07	1.57	0.01	0.07	0.01
	2253	1.35	1.6	1.45	1.4	0.85	0.9	1.65	1.24	0	1.34
	2256	0.52	0.15	0.07	0.36	0.13	0.12	1.63	0.01	0.02	0.02

グループ	Group #1	グループ2	グループ3
2344	2344	2248	2253
2346	2250		
2349	2256		
2.5.1			
6.1.2			
6.4.1			
2248			
2250			
2253			
2256			

ヒストアゴニストエピトープグループ1及びグループ2及びグループ3に対するビオチン化MAbの結合を阻害するMAbの能力。2344及び2253はエピトープ1及び2を代表するMAbである。

アッセイ形式: sol trkC + 非標識MAb + ビオチン化MAb + SA-HRP
(1μg/ml) (50μg/ml) (1μg/ml) (1:1K)

【図６Ａ】

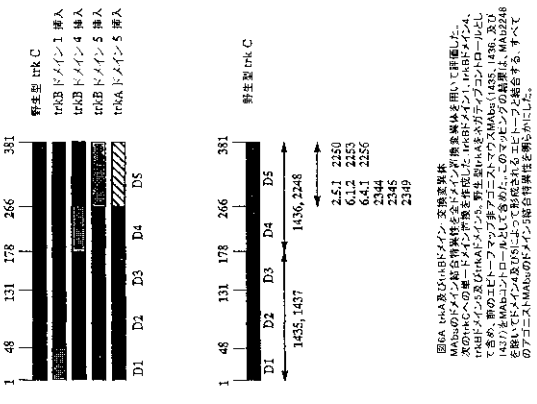
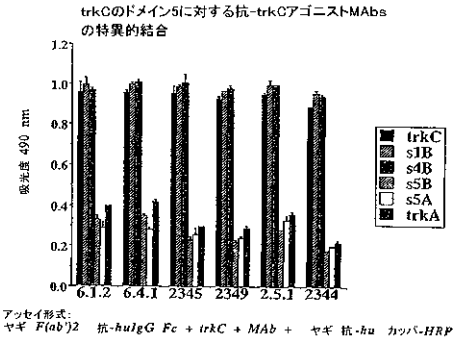
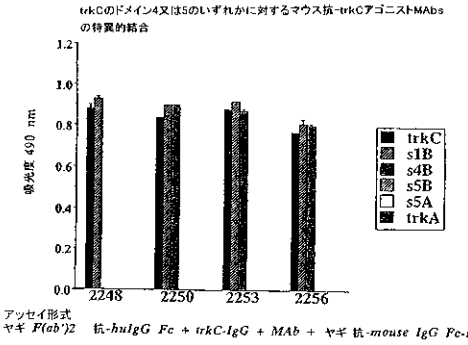


図6A trkA及びtrkBドメイン交換変異体
MAbエピトープのドメイン配置を評価した。
次のtrkCへの単一ドメイン置換を作成した。trkBドメイン1、trkBドメイン4、trkBドメイン5及びtrkAドメイン5。野生型trkA及びガラクトコントロールとして含む。前のエピトープマップ非アゴニストマウスMAbs(1435、1436、及び1437)及びMAbコントロールとして含めた。このマッピングの結果は、MAb2248を除いてドメイン4及び5によって形成されるエピトープと結合する。すべてのアゴニストMAbsのドメイン5結合特異性を明らかにした。

【図６Ｂ】



【図６Ｃ】



【図７】

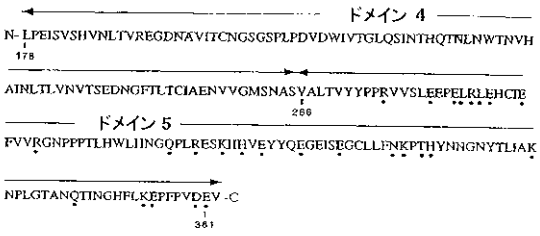


図7：hutrkCドメイン4及び5の配列。各々E,H,及びKへ変えられた残基L284、L286、E287を除いて、すべての点付き残基をアラニンへ変異させた(7)。

【図８】



【図９】

抗-trkCアゴニストMAbsの重鎖配列

CDR1 CDR2

2250 : N-QVQLQQSGAELMQPGASVKISCKSTGYTFSNFWIEWVKQRPG--HGLEWIGEILPGSDNTNYNEKFKG
2253 : N-QVQLQQSGAELMQPGASVKISCKSTGYTFSNFWIEWFKQRPG---HGLEWIGEILPGSDNTNYNEKFKG
2256 : N-QVQLQQPGAELVKPGASVKLSCKASGYTFTSYWMHWVKQRPG---QGLEWIGEIYPSNGRINYNEKFKS

6.1.2: N-QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGGYYWSWIRQHPGKGLEWIGYIYYSG-STNYNPSLKS
6.4.1: N-QVQLQESGPGLVRPSETLSLTCTVSGG--SISTYYWNWIRQPAGKGLEWIGRIYTSG-STNYNPSLKS
2345 : N-QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGGYYWSWIRQHPEKGLEWIGYIFYSG-RTYYNPSLKS
2349 : N-QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGYYYWSWIRQHPGKGLEWIGYIYYSG-STYYNPSLKS

CDR3

2250 : KATFTADTSSNTAYMQLSSLTSEDSAVYYCARKN----RN----YYGNYYYWGQGTLVTVSA-C
2253 : KATFTADTSSNTAYMQLSSLTSEDSAVYYCARKN----RN----YYGNIVVWGAGTTLTVSS-C
2256 : KATLTVDKSSSTAYMQLSSLTSEDSAVYYCARRY----YYGNSYFAWTFDVWGAGTTLTVSS-C

6.1.2: RVTISVDTSKNQFSLKLSSVTAADTAVYYCTRDRDYDSFGDYTSYYGMDVWGQGTTVTVSS-C
6.4.1: RVTMSVDTSKNQFSLKLSSVTAADTAVYYCARDGIGSNPFD---------WGQGTLVTVSS-C
2345 : RVTISVDTSKNQFSLKLNSVTAADTAVYYCARER-IAAAGADYYYNGLDVWGQGTTVTVSS-C
2349 : RLTISVDTSKNQFSLKLSSVTAADTAVYYCARER-IAAAGTDYYYNGLAVWGQGTTVTVSS-C

【図１０】

抗-trkCアゴニストMAbsの軽鎖配列

CDR1 CDR2

2250 : N-DIVMTQSPASLAVSLGQRATISYRASKSVSTSG--YSYMHWNQQKPGQPPRLLIYLVSNLESGVPARF
2253 : N-QIVLTQSPAIMSASPGEKVTITCSASSSVSY------MDWFQQKPGTSPKLWIYSTSNLASGVPARF
2256 : N-DIVLTQSPASLAVSLGQRATISCRASESVDNYG--ISFMNWFQQKPGQPPKLLIYAASNQGSGVPARF

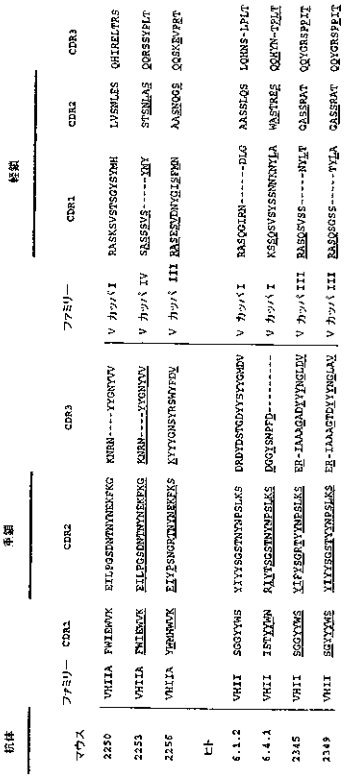
6.1.2: N-DIQMTQSPSSLSASVGDRVTITCRASQGIRN------DLGWYQQKPGKAPKRLIYAASSLQSGVPSRF
6.4.1: N-DIQMTQSPDSLAVSLGERATINCKSSQSVSYSSMNKNYLAWYQQKPGQPPKLLIYWASTRESGVPDRI
2345 : N-EIVLTQSPGTLSLSPGERATLSCRASQSVSS-----NYLAWYQQKPGQAPRLLIYGASSRATGIPDRF
2349 : N-GIVLTQSPGTLSLSPGERATFSCRASQSGSS-----TYLAWYQQKPGQAPRLIYYGASSRATGIPDRF

CDR3

2250 : SGSGSGTDFTLNIHPVEEDAATYYCQHIRE-LTRSARGQSWKKR---C
2253 : SGSGSGTSYSLTISRMEADDAATYYCQQRSS-YPLTFGAGTKLELKR-C
2256 : SGSGSGTDFSLNIHPMEEDDTAMYFCQQSKE-VPRTFGGGTKLEMKR-C

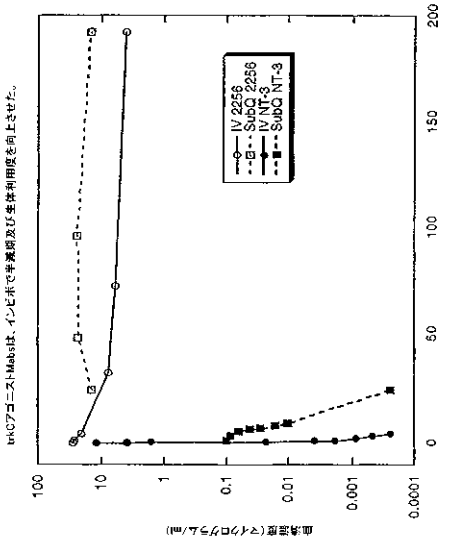
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6.4.1: SGSGSGTDFTLTISSLQAEDVAVYYCQQHYN-TPLTFGGGTKVEIKR-C
2345 : SGSGSGTDFTLTISRLEPEDFAVYYCQQYGRSPPTFGQGTRLEIKR-C
2349 : SGSGSGTDFTLTISRLEPEDFAVYYCQQYGRSPPTFGQGTRLEIKR-C

【図１１】

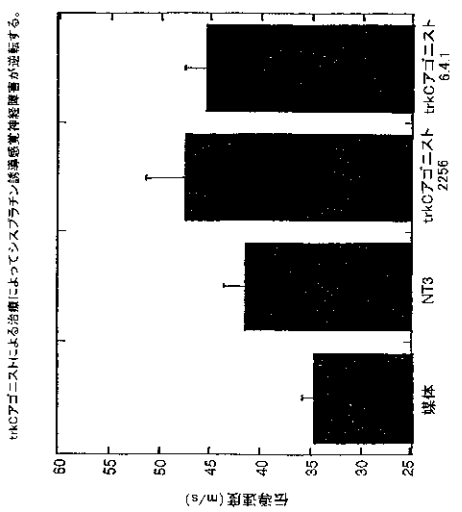


抗体		重鎖				軽鎖			
	ファミリー	CDR1	CDR2	CDR3	ファミリー	CDR1	CDR2	CDR3
マウス								
2250	VHIIA	FWISWVK	EILPGSGNTNYNEKFKG	KNRN----YYGNYVV	V カッパ I	RASKSVSTSGYSYMH	LVSNLES	QHIRELTRS
2253	VHIIA	FWISWVK	EILPGSGNTNYNEKFKG	KNRN----YYGNYVV	V カッパ IV	SASSSVS-----YMY	STSNLAS	QQRSSYPLT
2256	VHIIA	YGMHWVK	EIYPSNGRTDYNEKFKS	KYYYGNSYRSWYFDV	V カッパ III	RASESVDNYGISFMN	AASNQGS	QQSKEVPWT
ヒト								
6.1.2	VHII	SGGYYWS	YIYYSGSTNYNPSLKS	DRDTDSTGDYYYGMDV	V カッパ I	RASQGIRN-----DLG	AASSLQS	LQDNS-YPLT
6.4.1	VHII	ISFYYDGN	RLYTSGSTNYNPSLKS	DGGYSMFFD---------	V カッパ I	KSSQSVYSSSNNKNYLA	WASTRES	QQYYS-TPLT
2345	VHII	SGGYYWS	YIFYSGFTYYNPSLKS	ER-IAAAQNDYYNGLDV	V カッパ III	RASQSVSS-----NYLT	GASRRAT	QQYGRSPPIT
2349	VHII	SHYYWS	YIYYSGSTYYNPSLKS	ER-IAAATDIYYNGLAV	V カッパ III	RASQSGSS-----YYLA	GASSRAT	QQYGRSPPIT

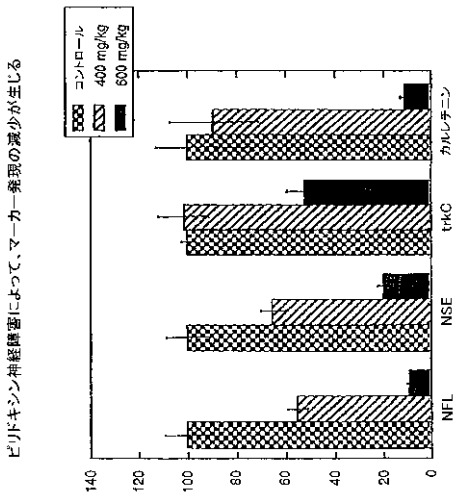
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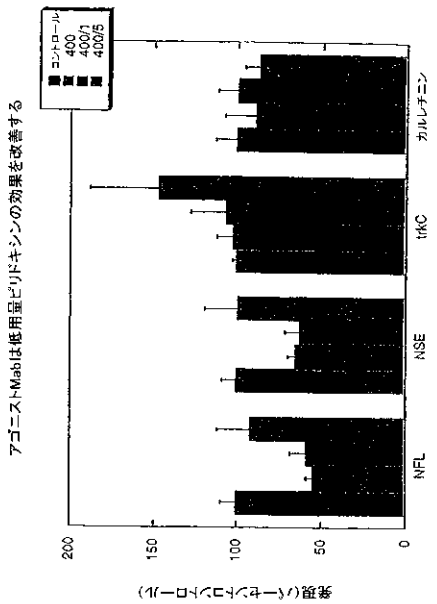
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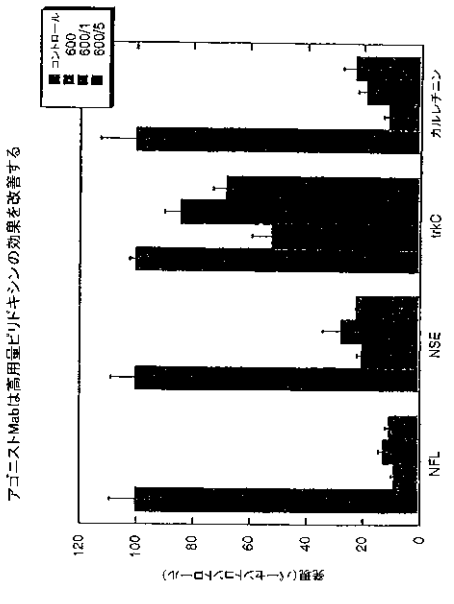
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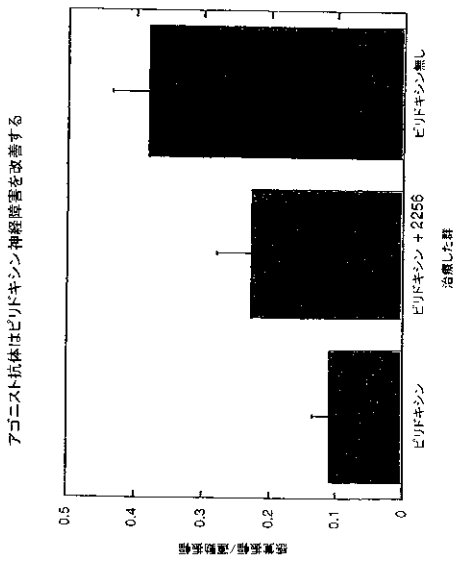
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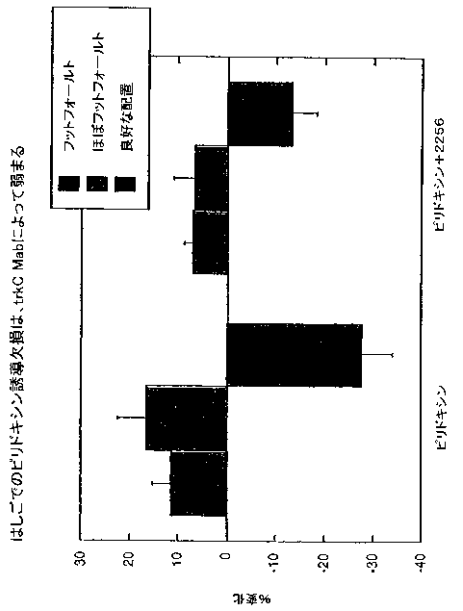
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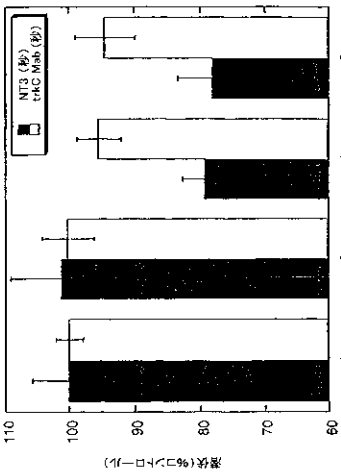
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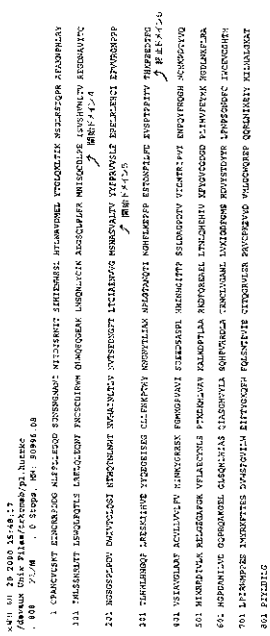
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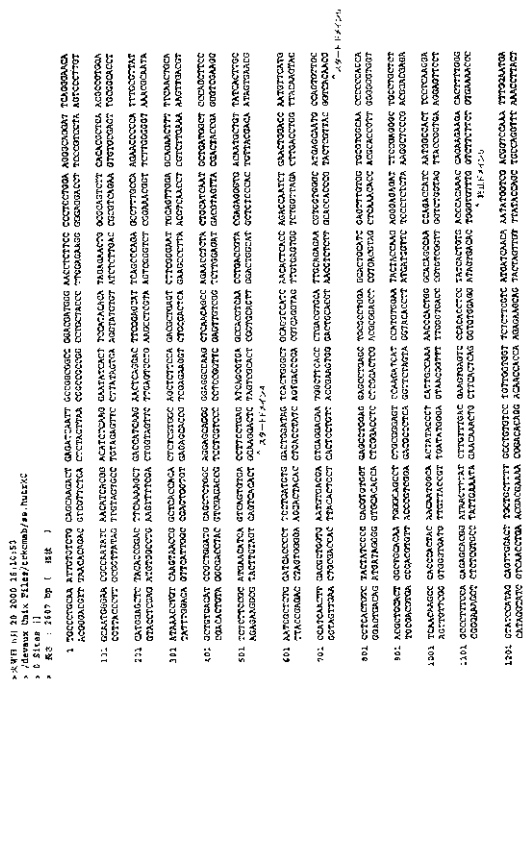
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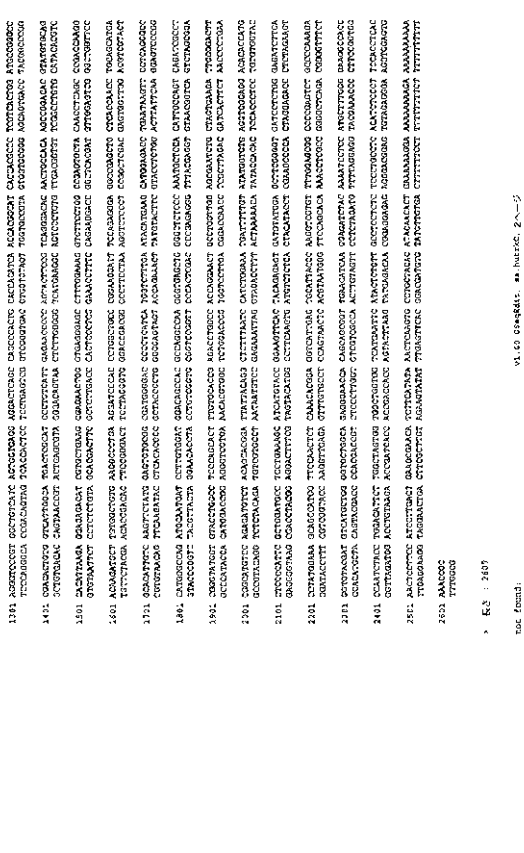
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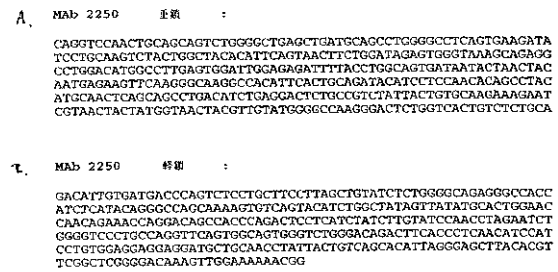
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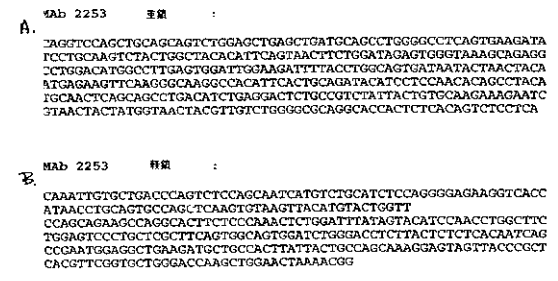
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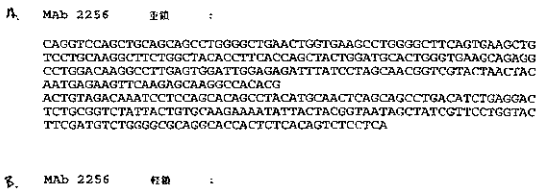
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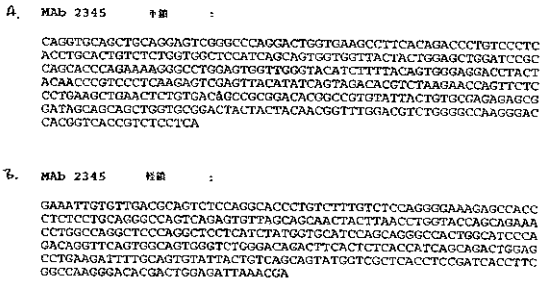
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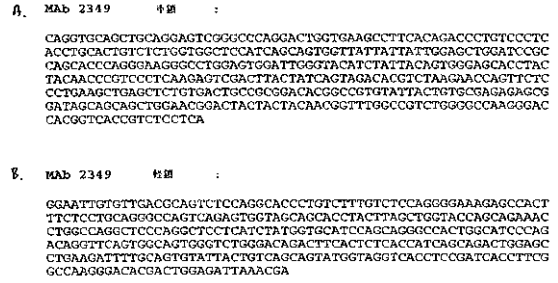
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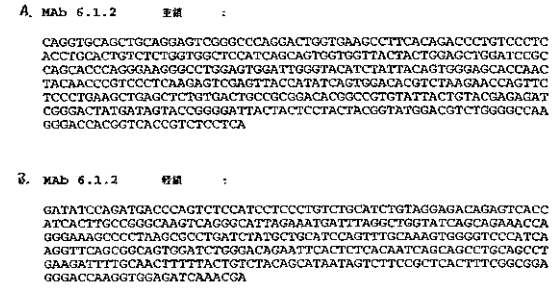
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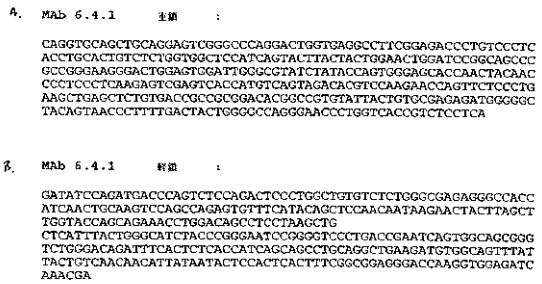
【図２６】



【図２７】



【図２８】



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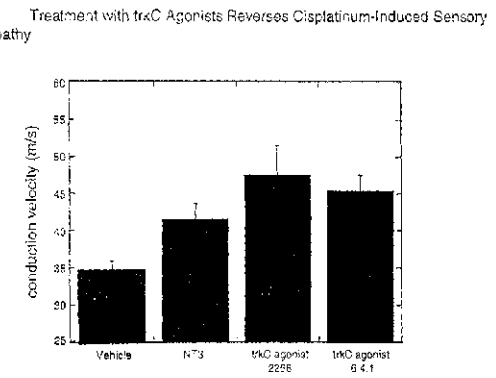
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[Continued on next page]

(54) Title: AGONIST ANTI TRK C MONOCLONAL ANTIBODIES



(57) Abstract: The invention concerns agonist anti-trkC monoclonal antoibodies which mimic certain biological activities of NT-3, the native ligand of trkC. The invention further concerns the use of such antibodies in the prevention and/or treatment of cellular degeneration, including nerve cell damage associated with acute nervous cell system injury and chronic neurodegenerative diseases, including peripheral neuropathy.

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AGONIST ANTI-TRK-C MONOCLONAL ANTIBODIES

Background of the Invention

Field of the invention

This invention concerns agonist anti-trkC monoclonal antibodies. It further concerns the use of the agonist antibodies in the prevention and/or treatment of cellular degeneration, including nerve cell damage associated with acute nervous cell system injury and chronic neurodegenerative diseases, including peripheral neuropathy.

Description of the Related Art

Neurotrophins are a family of small, basic proteins, which play a crucial role in the development and maintenance of the nervous system. The first identified and probably best understood member of this family is nerve growth factor (NGF), which has prominent effects on developing sensory and sympathetic neurons of the peripheral nervous system (Levi-Montalcini, R. and Angeletti, P.U., Physiol. Rev. 48, 534-569 [1968]; Thoenen, H. *et al.*, Rev. Physiol. Biochem. Pharmacol. 109, 145-178 [1987]). Although NGF had been known for a long time, including a homolog from the mouse submandibular gland, the mature, active form of which is often referred to as - or 2.5S NGF, it was only many years later that sequentially related but distinct polypeptides with similar functions were identified.

The first in line was a factor called brain-derived neurotrophic factor (BDNF), which was cloned and sequenced by Leibrock, J. *et al.* (Nature 341, 149-152 [1989]). This factor was originally purified from pig brain (Barde, Y.A. *et al.*, EMBO J. 1, 549-553 [1982]), but it was not until its cDNA was cloned and sequenced that its homology with NGF became apparent. The overall amino acid sequence identity between NGF and BNDF is about 50%. In view of this finding, Leibrock et al. speculated that there was no reason to think that BDNF and NGF should be the only members of a family of neurotrophins having in common structural and functional characteristics.

Indeed, further neurotrophins closely related to -NGF and BDNF have since been discovered. Several groups identified a neurotrophin originally called neuronal factor (NF), and now referred to as neurotrophin-3 (NT-3) (Ernfors *et al.*, Proc. Natl. Acad. Sci. USA 87, 5454-5458 [1990]; Hohn *et al.*, Nature 344, 339 [1990]; Maisonpierre *et al.*, Science 247, 1446 [1990]; Rosenthal *et al.*, Neuron 4, 767 [1990]; Jones and Reichardt, Proc. Natl. Acad. Sci. USA 87, 8060-8064 [1990]; Kaisho *et al.*, FEBS Lett. 266, 187 [1990]). NT-3 shares about 50% of its amino acids with both -NGF and BDNF (NT-2). Neurotrophins-4 and -5 (NT-4 and NT-5), have been added to the family (U.S. Patent No. 5,364,769 issued November 15, 1994; Hallbook, F. *et al.*, Neuron 6, 845-858 [1991]; Berkmeier, L.R. *et al.*, Neuron 7, 857-866 [1991]; Ip *et al.*, Proc. Natl. Acad. Sci USA 89, 3060-3064 [1992]). The mammalian molecule initially described by Berkmeier *et al. supra*, which was subsequently seen to be the homolog of Xenopus NT-4, is usually referred to as NT-4/5. In addition, there is an acidic homologous protein described in mammals which is referred to as NT-6 (Berkemeir, *et al.*, Somat. Cell Mol. Genet. 18(3):233-245 [1992]). More recently, another homologus protein from the fish, *Xiphophorus* has also been labeled NT-6 (Gotz *et al.*, Nature 372:266-269 [1994]). There are two proteins described in the literature as NT-7, one cloned from the carp, *Cyprinus*, (Lai, *et al.*, Mol. Cell. Neurosci. 11[1-

2):64-76 (1998)) and one from the zebrafish, *Danio* (Nilsson *et al.*, FEBS Letters 424(3):285-90 (1998)). None of these last three described fish neurotrophins has been described outside fish, and their relationship to any mammalian neurotrophins is unclear. The amino acid sequence of zebrafish neurotrophin-7 (zNT-7) is more closely related to that of fish nerve growth factor (NGF) and neurotrophin-6 (NT-6) than to that of any other neurotrophin. zNT-7 is, however, equally related to fish NGF and NT-6 (65% and 63% amino acid sequence identity, respectively) indicating that it represents a distinct neurotrophin sequence. zNT-7 contains a 15 amino acid residue in a beta-turn region in the middle of the mature protein. Recombinant zNT-7 was able to bind to the human p75 neurotrophin receptor and to induce tyrosine phosphorylation of the rat trkA receptor tyrosine kinase, albeit less efficiently than rat NGF. zNT-7 did not interact with rat trkB or trkC, indicating a similar receptor specificity as NGF. We propose that a diversification of the NGF subfamily in the neurotrophin evolutionary tree occurred during the evolution of teleost fishes which in the appearance of several additional members, such as zNT-7 and NT-6, is structurally and functionally related to NGF.

Neurotrophins, similarly to other polypeptide growth factors, affect their target cells through interactions with cell surface receptors. According to our current knowledge, two kinds of transmembrane glycoproteins serve as receptors for neurotrophins. Equilibrium binding studies have shown that neurotrophin-responsive neurons possess a common low molecular weight (65-80 kDa), low affinity receptor (LNGFR), also termed as $p75^{NTR}$ or p75, which binds NGF, BDNF, and NT-3 with a K_d of 2×10^{-9} M, and large molecular weight (130-150 kDa), high affinity (K_d in the 10^{-11} M) receptors, which are members of the trk family of the receptor tyrosine kinases.

The first member of the trk receptor family, trkA, was initially identified as the result of an oncogenic transformation caused by the translocation of tropomyosin sequences onto its catalytic domain (Martin-Zanca *et al.*, Mol. Cell. Biol. 9(1):24-33 (1989)). Later work identified trkA as a signal transducing receptor for NGF. Subsequently, two other related receptors, mouse and rat trkB (Klein *et al.*, EMBO J. 8, 3701-3709 (1989); Middlemas *et al.*, Mol. Cell. Biol. 11, 143-153 (1991); EP 455,460 published 6 November 1991) and porcine, mouse and rat trkC (Lamballe *et al.*, Cell 66, 967-979 (1991); EP 522,530 published 13 January 1993), were identified as members of the trk receptor family. The structures of the trk receptors are quite similar, but alternate splicing increases the complexity of the family by giving rise to two known forms of trkA, three known forms of trkB (two without functional tyrosine kinase domains) and at least four forms of trkC (several without functional tyrosine kinase domain, and two with small inserts in the tyrosine kinase domain).

The role of the p75 and trk receptors is controversial. It is generally accepted that trk receptor tyrosine kinases play an important role in conferring binding specificity to a particular neurotrophin, however, cell lines expressing trkA bind not only NGF but also NT-3 and NT-4/5 (but not BDNF), trkB expressing cells bind BDNF, NT-3, NT-4, and NT-4/5 (but not NGF), in contrast to trkC-expressing cells which have been reported to bind NT-3 alone (but not the other neurotrophins). Furthermore, it has been shown in model systems that the various forms of trk receptors, arising from alternate splicing events, can activate different intracellular signalling pathways, and therefore presumably mediate different physiological functions in vivo. It is unclear whether cells expressing a given trk receptor

in the absence of p75 bind neurotrophins with low or high affinity (Meakin and Shooter, Trends Neurosci. 15, 323-331 [1992]).

Published results of studies using various cell lines are confusing and suggest that p75 is either essential or dispensable for neurotrophin responsiveness. Cell lines that express p75 alone bind NGF, BDNF, NT-3, and NT-4 with similar low affinity at equilibrium, but the binding rate constants are remarkably different. As a result, although p75-binding is a common property of all neurotrophins, it has been suggested the p75 receptor may also play a role in ligand discrimination (Rodriguez-Tebar et al., EMBO J. 11, 917-922 [1992]). While the trk receptors have been traditionally thought of as the biologically significant neurotrophin receptors, it has recently been demonstrated that in melanoma cells devoid of trkA expression, NGF can still elicit profound changes in biological behavior presumably through p75 (Herrmann *et al.*, Mol. Biol. Cell 4, 1205-1216 [1993]). Davies *et al.* (Neuron 11, 565-574 [1993]) reported the results of studies investigating the role of p75 in mediating the survival response of embryonic neurons to neurotrophins in a model of transgenic mice carrying a null mutation in the p75 gene. They found that p75 enhances the sensitivity of NGF-dependent cutaneous sensory neurons to NGF. There have now been many studies showing that p75 is capable of mediating at least some of the biological effects of the neurotrophins. The field is still somewhat controversial, but p75 signaling has been implicated in controlling cell death, and neurite outgrowth. (Barker, PA, Cell Death Diff. 5:346-356 [1998]; Bradeson *et al.*, Cell Death Diff. 5:357-364 [1998]; Casaccia-Bonnefil, *et al.*, Cell Death Diff. 5:357-364 [1998]; Raoul *et al.*, Curr. Op. Neurobiol. 10:111-117 [2000]; Davies, AM, Curr. Biol. 10:R198-R200 [2000]). Importantly, stimulation of p75 has been shown to modify the effects of stimulating trkC (Hapner, *et al.*, Developm. Biol. 201:90-100 [1998]).

The extracellular domains of full-length native trkA, trkB and trkC receptors have five functional domains, that have been defined with reference to homologous or otherwise similar structures identified in various other proteins. The domains have been designated starting at the *N*-terminus of the amino acid sequence of the mature trk receptors as 1) a first cysteine-rich domain extending from amino acid position 1 to about amino acid position 32 of human trkA, from amino acid position 1 to about amino acid position 36 of human trkB, and from amino acid position 1 to about amino acid position 48 of human trkC; 2) a leucine-rich domain stretching from about amino acid 33 to about amino acid to about amino acid 104 in trkA; from about amino acid 37 to about amino acid 108 in trkB, and from about amino acid 49 to about amino acid 120 in trkC; 3) a second cysteine-rich domain from about amino acid 105 to about amino acid 157 in trkA; from about amino acid 109 to about amino acid 164 in trkB; and from about amino acid 121 to about amino acid 177 in trkC; 4) a first immunoglobulin-like domain stretching from about amino acid 176 to about amino acid 234 in trkA; from about amino acid 183 to about amino acid 239 in trkB; and from about amino acid 196 to about amino acid 257 in trkC; and 5) a second immunoglobulin-like domain extending from about amino acid 264 to about amino acid 330 in trkA; from about amino acid 270 to about amino acid 334 in trkB; and from about amino acid 288 to about amino acid 351 in trkC.

Neurotrophins exhibit actions on distinct, but overlapping, sets of peripheral and central neurons. These effects range from playing a crucial role in ensuring the survival of developing neurons (NGF in sensory and

sympathetic neurons) to relatively subtle effects on the morphology of neurons (NT-3 on purkinje cells). These activities have led to interest in using neurotrophins as treatments of certain neurodegenerative diseases. NT-3 has also been found to promote proliferation of peripheral blood leukocytes and, as a result, it has been suggested that NT-3 can be used in the treatment of neutropenia, infectious disease and tumors (U.S. Patent No. 6,015,552 issued on June 18, 2000).

The roles of neurotrophins in regulating cardiovascular development and modulating the vascular response to injury have also been investigated (Donovan *et al.*, Nature Genetics 14:210-213 [1996]; Donovan *et al.*, A.J. Path. 147:309-324 [1995]; Kraemer *et al.*, Arteriol. Thromb. and Vasc. Biol. 19:1041-1050 [1999]). Neurotrophins have been described as potential therapeutics for regulating angiogenesis and vascular integrity (PCT Publication WO 00/24415, published May 4, 2000).

Despite their promise in the treatment of cellular degeneration, such as occurs due to neurodegenerative disease and acute neuronal injuries, and potentially angiogenesis, neurotrophins have several shortcomings. One significant shortcoming is the lack of specificity. Most neurotrophins cross-react with more than one receptor. For example NT-3, the preferred ligand of the trkC receptor tyrosine kinase, also binds to and activates trkA and trkB (Barbacid, J. Neurobiol. 25:1386-1403 [1994]; Barbacid, Ann. New York Acad. Sci. 766:442-458 [1995]; Ryden and Ibanez, J. Biol. Chem. 271:5623-5627 [1996]; Belliveau *et al.*, J. Cell. Biol. 136:375-388 [1997]; Farinas *et al.*, Neuron 21:325-334 [1998]). As a result, it is difficult to devise therapies that target a specific population of neurons. Another limitation of neurotrophin therapy is that neurotrophins, including NT-3 are known to elicit hyperalgesia (Chaudhry, *et al.*, Muscle and Nerve 23:189-192 [2000]). In addition, some neurotrophins such as NT-3 have poor pharmacokinetic and bioavailability properties in rodents, which raise serious questions about their human clinical applications (Haase *et al.*, J. Neurol. Sci. 160:S97-S105 [1998], dosages used in Helgren *et al.*, J. Neurosci. 17(1):372-82 [1997], and data below).

Accordingly, there is a great need for the development of new therapeutic agents for the treatment of neurodegenerative disorders and acute nerve cell injuries that are devoid of the known shortcomings of neurotrophins.

Summary of the Invention

The current invention is based on the development and characterization of agonist anti-trkC monoclonal antibodies, directed against epitopes in the extracellular domain of trkC receptor, which mimic the biological activities of NT-3, the natural ligand of trkC receptor but are free of some of the known detriments of NT-3. The invention also demonstrates the usefulness of these agonist antibodies in the treatment of neuropathy in an experimental animal model. Anti-trkC agonist antibodies offer numerous advantages over NT-3 in prophylactic or therapeutic treatment of cellular degeneration, such as nerve cell damage, in particular nerve cell injury associated with neurodegenerative diseases, such as peripheral neuropathies or due to external factors, such as trauma, toxic agents, surgery, just to mention a few.

In one aspect, the invention concerns an agonist anti-trkC monoclonal antibody which

(a) shows no significant cross-reactivity with trkA or trkB; and

(b) recognizes an epitope in domain 5 of trkC.

Certain agonist antibodies of the present invention may additionally recognize an epitope in domain 4 of trkC. In a preferred embodiment, the antibodies bind both human and rodent (e.g. rat or mouse) trkC, and may be murine, chimeric (including humanized) or human antibodies. The antibodies mimic at least one activity of the native trkC ligand, NT-3, and may thus be effective in the prevention and/or treatment of various diseases involving cellular degeneration, including, for example, neuropathies, such as cisplatin- or pyridoxine-induced neuropathy, or diabetic neuropathy, and (where cellular degeneration involves bone marrow cell degeneration) disorders of insufficient blood cells, such as leukopenias including eosinopenia and/or basopenia, lymphopenia, monocytopenia, and neutropenia. In a particularly preferred embodiment, the agonist antibodies of the present invention show superior properties over NT-3, for example, do not cause hyperalgesia when administered to a patient, have increased bioavailability and/or higher specific activity as compared to NT-3.

In another aspect, the invention concerns an anti-trkC antibody heavy chain comprising the following CDR's: a CDR1 selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4 and 5; a CDR2 selected from the group consisting of SEQ ID NOs: 6, 7, 8, 9, 10 and 11; and a CDR3 selected from the group consisting of SEQ ID NOs: 12, 13, 14, 15, 16 and 17.

In yet another aspect, the invention concerns an anti-trkC antibody light chain comprising the following CDR's: a CDR1 selected from the group consisting of SEQ ID NOs: 18, 19, 20, 21, 22, 23 and 24; a CDR2 selected from the group consisting of SEQ ID NOs: 25, 26, 27, 28, 29 and 30; and a CDR3 selected from the group consisting of SEQ ID NOs: 31, 32, 33, 34, 35 and 36.

In a further aspect, the invention concerns a murine anti-trkC antibody heavy chain comprising the following CDR's:

(a) a CDR1 of the formula XaaWXaaXaaWVK (SEQ ID NO: 37), wherein Xaa at position 1 is F or Y; Xaa at position 3 is I or M; and Xaa at position 4 is E or K;

(b) a CDR2 of the formula EIXaaPXaaXaaXaaXaaTNYNEKFKXaa (SEQ ID NO: 38), wherein Xaa at position 3 is L or Y; Xaa at position 5 is G or S; Xaa at position 6 is S or N; Xaa at position 7 is D or G; Xaa at position 8 is N or R and Xaa at position 16 is G or S; and

(c) a CDR3 of the formula KNRNYYGNYVV (SEQ ID NO: 12) or KYYYGNSYRSWYFDV (SEQ ID NO:13).

In a still further aspect, the invention relates to a human anti-trkC antibody heavy chain comprising the following CDR's:

(a) a CDR1 of the formula XaaXaaXaaYYWXaa (SEQ ID NO: 39), wherein Xaa at position 1 is S or I; Xaa at position 2 is G or S; Xaa at position 3 is G, T or Y, and Xaa at position 7 is S or N;

(b) a CDR2 of the formula XaaIXaaXaaSGSXaaTXaaNPSLKS (SEQ ID NO: 40), wherein Xaa at position 1 is Y or R; Xaa at position 3 is Y or F; Xaa at position 4 is Y or T; Xaa at position 8 is S or R; and Xaa at position 10 is N or Y; and

(c) a CDR3 of the formula selected from the group consisting of DRDYDSTGDYYSYYGMDV (SEQ ID NO: 14); DGGYSNPFD (SEQ ID NO: 15); ERIAAAGXaaDYYYNGLXaaV (SEQ ID NO: 41), wherein Xaa at position 8 is A or T and Xaa at position 16 is D or A.

In another aspect, the invention concerns an anti-trkC agonist monoclonal antibody comprising a heavy chain comprising the CDR's of the murine anti-trkC antibody heavy chain of claim 14 associated with a light chain. The antibody preferably is human or comprises human framework residues, and preferably shows no significant cross-reactivity with trkA or trkB. Throughout the application, antibodies are defined in the broadest sense, and specifically include antibody fragment, such as an Fv fragment, Fab fragment, Fab' or $F(ab')_2$ fragment. Antibodies of all classes and isotypes are included, but IgG, in particular IgG-2 and IgG-4 are preferred.

In yet another aspect, the invention concerns isolated nucleic acid encoding a murine or human anti-trkC agonist antibody heavy or light chain, or a fragment thereof. In a specific embodiment, the nucleic acid is a nucleic acid molecule deposited with ATCC on June 21, 2000 under an accession number selected from the group consisting of PTA-2133, PTA-2134, PTA-2135, PTA-2136, PTA-2137, PTA-2138, PTA-2139, PTA-2140, PTA-2141, PTA-2142 and PTA-2143.

In a further aspect, the invention concerns a vector comprising a nucleic acid molecule encoding an antibody heavy and/or light chain as hereinabove defined. The invention also concerns cells transformed with such nucleic acid. The invention further concerns hybridoma cell lines transformed with such nucleic acid and antibodies produced by such hybridoma cells.

In a still further aspect, the invention concerns a pharmaceutical composition comprising an effective amount of an agonist anti-trkC monoclonal antibody as hereinabove defined in admixture with a pharmaceutically acceptable carrier.

In another aspect, the invention concerns a method for treating a disease or condition involving cell degeneration, comprising administering to a mammal an effective amount of an agonist anti-trkC antibody disclosed herein.

In yet another aspect, the invention concerns a method for treating a neuropathy or neurodegenerative disease, or repairing a damaged nerve cell comprising administering to a mammal an effective amount of an agonist anti-trkC antibody disclosed herein. The neuropathy may, for example, be a peripheral neuropathy, including, without limitation, diabetic neuropathy and large-fiber sensory neuropathies. The neurodegenerative disease may, for example, be amyotrophic lateral sclerosis (ALS), Alzheimer's disease, Parkinson's disease, Huntington's disease. The damaged neurons may be peripheral, such as sensory, e.g. dorsal root ganglia neurons, motor neurons, e.g. neurons from the spinal cord, or central neurons, and the injury may be due to a variety of external and internal factors, including trauma, exposure to neurotoxins, metabolic diseases, infectious agents, etc.

In a further aspect, the invention concerns a method for promoting the development, proliferation, maintenance or regeneration of peripheral neurons, comprising contacting such neurons with an effective amount of an antibody of the present invention.

In a still further aspect, the invention concerns a method for the treatment (including prevention) of a disease or condition involving cell degeneration in a mammalian subject by introducing nucleic acid encoding an anti-trkC antibody herein into a cell of such subject. The method (gene therapy) preferably concerns the treatment of a neuropathy or neurodegenerative disease, or reparation of a damaged nerve cell. Accordingly, the recipient cells preferably are nerve cells.

In yet another aspect, the invention concerns delivery vehicles containing genetic material (nucleic acid) encoding an anti-trkC antibody suitable for gene therapy use.

In an additional aspect, the invention concerns a method of inducing angiogenesis by delivering an anti-trkC antibody of the present invention in an amount effective to induce angingenesis. The delivery specifically includes the administration of the antibodies and the delivery of nucleic acid encoding the antibodies (e.g. in gene therapy).

In yet another aspect, the invention concerns an isolated nucleic acid molecule encoding a murine or human anti-trkC agonist antibody heavy or light chain selected from the group consisting of SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65; SEQ ID NO: 66; SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70 and SEQ ID NO: 71. The present invention also concerns a polypeptide encoded by one or more of the isolated nucleic acid molecules.

In another aspect, the invention concerns a whole cell transformed with nucleic acid encoding murine or human anti-trkC agonist antibody heavy chain, light chain or both heavy and light chain.

Brief Description of the Drawings

Figures 1A-D show agonist activity of various human (A and C) and murine (B and D) monoclonal antibodies against trkC receptor demonstrated using KIRA (A and B) and PC12 neurite outgrowth assay (C and D). Protein A purified monoclonal antibodies were diluted to 27 μg/ml in KIRA stimulation buffer (F12/DMEM 50:50 containing 2% bovine serum albumin [BSA, Intergen Co., Purchase, NY) and 25 mM Hepes, 0.2 μm filtered). The monoclonal antibodies were then diluted 1:3 (8 dilutions total; concentrations ranged from 0.01-180 nM Nab) in stimulation media. GD-transfected CHO cells (5×10^4 cells/well) were then stimulated with either NT-3 or Mab (dilutions assayed in duplicate) for 6 hours and the assay was completes as described in the examples (Fig. 1A, human Mabs; Fig. 1B, murine Mabs). The purified Mabs were assayed for agonist activity in the PC12 neurite outgrowth assay as described in the examples. Rat PC12 cells were transfected with full-length human trkC and the cells plated at a density of 1000 cells/well. Three days following transfection, the Mabs were added in triplicate (concentrations ranging from 0.0002 to 2.7 nM) to the wells containing the trkC transfectants and incubated for an additional 3 days at 37 °C. The cells were then analyzed by phase contrast microscopy and cells with neurites exceeding two-times the diameter of the cell were counted.

Figure 2 shows that agonist anti-trkC monoclonal antibodies bind specifically to trkC using 6.1.2 antibody as a representative example.

Figure 3 demonstrates that agonist anti-trkC monoclonal antibodies recognize human trkC more efficiently than rat trkC. The ability of the monoclonal antibodies to bind rat trkC was determined using an immunoadhesin construct of the receptor. TrkC (human trkC-gD or rat trkC-IgG) was immobilized on microtiter plates (100 μl of a 1 μg/ml solution diluted in 50 mM carbonate buffer, pH 9.5) overnight. The plates were washed and blocked. The Mabs were then diluted to 1 μg/ml in PBS containing 0.5% BSA and 0.05% Tween 20, added to the appropriate wells (100 μl/well), and incubated for one hour at room temperature. The plates were washed and the appropriate HRP conjugate was added (human Mabs: goat anti-human κ-HRP, 1:5K; murine Mabs: goat anti-molgG (Fc)-HRP, 1:5 K) and incubated for one hour at room temperature. The plates were then washed, developed and read.

Figure 4 shows a representative example of epitope mapping using competition ELISA. A biotinylated human anti-trkC 6.1.2 monoclonal antibody was incubated with immobilized trkC in the absence or presence of excess of various unlabeled anti-trkC monoclonal antibodies.

Figure 5 summarizes the results of epitope mapping using competition ELISA.

Figures 6A-C show a schematic diagram of various trkC chimera (A) and their use in mapping of epitopes on trkC recognized by various agonist human (B) and murine (C) anti-trkC monoclonal antibodies.

Figure 7 shows amino acid sequence of human trkC domain 4 and 5 showing residues that were targeted for mutagenesis to decipher their roles in recognition by agonist anti-trkC monoclonal antibodies.

Figure 8 shows 3-dimensional ribbon diagram of trkC in complex with anti-trkC monoclonal antibodies. Specifically shown are the amino acid residues of trkC that are likely to play an important role in recognition by CDRs of anti-trkC antibodies.

Figure 9 shows the amino acid sequence of the heavy chain variable (V_H) region from murine and human anti-trkC agonist monoclonal antibodies. In addition, the three CDR regions (CDR1, CDR2 and CDR3) are highlighted in bold. The amino acid sequence of CDR1 of the 2250 and 2253 heavy chain is SEQ ID NO: 1. The amino acid sequence of CDR1 of the 2256 heavy chain is SEQ ID NO: 2. The amino acid sequence of CDR1 of the 6.1.2 and 2345 heavy chain is SEQ ID NO: 3. The amino acid sequence of CDR1 of the 6.4.1 heavy chain is SEQ ID NO: 4. The amino acid sequence of CDR1 of the 2349 heavy chain is SEQ ID NO: 5. The amino acid sequence of CDR2 of the 2250 and 2253 heavy chain is SEQ ID NO: 6. The amino acid sequence of CDR2 of the 2256 heavy chain is SEQ ID NO: 7. The amino acid sequence of CDR2 of the 6.1.2 heavy chain is SEQ ID NO: 8. The amino acid sequence of CDR2 of the 6.4.1 heavy chain is SEQ ID NO: 9. The amino acid sequence of CDR2 of the 2345 heavy chain is SEQ ID NO: 10. The amino acid sequence of CDR2 of the 2349 heavy chain is SEQ ID NO: 11. The amino acid sequence of CDR3 of the 2250 and 2253 heavy chain is SEQ ID NO: 12. The amino acid sequence of CDR3 of the 2256 heavy chain is SEQ ID NO: 13. The amino acid sequence of CDR3 of the 6.1.2 heavy chain is SEQ ID NO: 14. The amino acid sequence of CDR3 of the 6.4.1 heavy chain is SEQ ID NO: 15. The amino acid sequence of CDR3 of the 2345 heavy chain is SEQ ID NO: 16. The amino acid sequence of CDR3 of the 2349 heavy chain is SEQ ID NO: 17.

Figure 10 shows the amino acid sequence of the light chain variable (V_L) region from murine and human anti-trkC agonist monoclonal antibodies. In addition, the three CDR regions (CDR1, CDR2 and CDR3) are highlighted in bold. The amino acid sequence of CDR1 of the 2250 light chain is SEQ ID NO: 18. The amino acid sequence of CDR1 of the 2253 light chain is SEQ ID NO: 19. The amino acid sequence of CDR1 of the 2256 light chain is SEQ ID NO: 20. The amino acid sequence of CDR1 of the 6.1.2 light chain is SEQ ID NO: 21. The amino acid sequence of CDR1 of the 6.4.1 light chain is SEQ ID NO: 22. The amino acid sequence of CDR1 of the 2345 light chain is SEQ ID NO: 23. The amino acid sequence of CDR1 of the 2349 light chain is SEQ ID NO: 24. The amino acid sequence of CDR2 of the 2250 light chain is SEQ ID NO: 25. The amino acid sequence of CDR2 of the 2253 light chain is SEQ ID NO: 26. The amino acid sequence of CDR2 of the 2256 light chain is SEQ ID NO: 27. The amino acid sequence of CDR2 of the 6.1.2 light chain is SEQ ID NO: 28. The amino acid sequence of CDR2 of the 6.4.1 light chain is SEQ ID NO: 29. The amino acid sequence of CDR2 of the 2345 and 2349 light chain is SEQ ID NO: 30. The amino acid sequence of CDR3 of the 2250 light chain is SEQ ID NO: 31. The amino acid sequence of CDR3 of the 2253 light chain is SEQ ID NO: 32. The amino acid sequence of CDR3 of the 2256 light chain is SEQ ID NO: 33. The amino acid sequence of CDR3 of the 6.1.2 light chain is SEQ ID NO: 34. The amino acid sequence of CDR3 of the 6.4.1 light chain is SEQ ID NO: 35. The amino acid sequence of CDR3 of the 2345 and 2349 light chain is SEQ ID NO: 36.

Figure 11 shows amino acid sequence of CDRs of heavy and light variable chains of murine and human anti-trkC agonist monoclonal antibodies. Also shown are the families to which these sequences belong based on homology with CDR sequences available in databases.

Figure 12 shows that anti-trkC agonist monoclonal antibodies have improved half-life and bioavailability *in vivo*.

Figure 13 shows effect of anti-trkC agonist monoclonal antibodies on cisplatin-induced neuropathy.

Figure 14 shows decrease in marker expression caused by pyridoxine neuropathy.

Figure 15 shows amelioration of the effects of low doses of pyridoxine by agonist anti-trkC monoclonal antibodies.

Figure 16 shows amelioration of the effects of high doses of pyridoxine by agonist anti-trkC monoclonal antibodies.

Figure 17 shows amelioration of pyridoxine-neuropathy by an anti-trkC agonist monoclonal antibody.

Figure 18 shows attenuation of pyridoxine-induced deficit of ladder by agonist anti-trkC monoclonal antibodies.

Figure 19 shows that NT3, but not anti-trkC agonist monoclonal antibodies, causes hyperalgesi at therapeutic doses.

Figure 20 shows the amino acid sequence of human trkC receptor (SEQ ID NO: 56) where the boundaries of domains 4 and 5 are indicated.

Figure 21 (in 2 pages) shows the nucleotide sequence of human trkC receptor (SEQ ID NO: 57).

Figure 22 shows the nucleotide sequence of the heavy chain (A; SEQ ID NO: 58) and light chain (B; SEQ ID NO: 59) of the anti-trkC agonist monoclonal antibody 2250.

Figure 23 shows the nucleotide sequence of the heavy chain (A; SEQ ID NO: 60) and light chain (B; SEQ ID NO: 61) of the anti-trkC agonist monoclonal antibody 2253.

Figure 24 shows the nucleotide sequence of the heavy chain (A; SEQ ID NO: 62) and light chain (B; SEQ ID NO: 63) of the anti-trkC agonist monoclonal antibody 2256.

Figure 25 shows the nucleotide sequence of the heavy chain (A; SEQ ID NO: 64) and light chain (B; SEQ ID NO: 65) of the anti-trkC agonist monoclonal antibody 2345.

Figure 26 shows the nucleotide sequence of the heavy chain (A; SEQ ID NO: 66) and light chain (B; SEQ ID NO: 67) of the anti-trkC agonist monoclonal antibody 2349.

Figure 27 shows the nucleotide sequence of the heavy chain (A; SEQ ID NO: 68) and light chain (B; SEQ ID NO: 69) of the anti-trkC agonist monoclonal antibody 6.1.2.

Figure 28 shows the nucleotide sequence of the heavy chain (A; SEQ ID NO: 70) and light chain (B; SEQ ID NO: 71) of the anti-trkC agonist monoclonal antibody 6.4.1.

Detailed Description of the Preferred Embodiment

A. Definitions

The term "neurotrophin" and its grammatical variants are used interchangeably, and refer to a family of polypeptides comprising nerve growth factor (NGF) and sequentially related homologs. NGF, brain-derived growth factor (BDNF, a.k.a. NT-2), neurotrophin-3 (NT-3), neurotrophins-4 and -5 (NT-4/5), neurotrophin-6 (NT-6), and neurotrophin-7 (NT-7) have so far been identified as members of this family.

The term "neurotrophin" includes native neurotrophins of any (human or non-human) animal species, and their functional derivatives, whether purified from a native source, prepared by methods of recombinant DNA technology, or chemical synthesis, or any combination of these or other methods. "Native" or "native sequence" neurotrophins have the amino acid sequence of a neurotrophin occurring in nature in any human or non-human animal species, including naturally-occurring truncated and variant forms, and naturally-occurring allelic variants.

The terms "trk", "trk polypeptide", "trk receptor" and their grammatical variants are used interchangeably and refer to polypeptides of the receptor tyrosine kinase superfamily, which are capable of binding at least one native neurotrophin. Currently identified members of this family are trkA ($p140^{trkA}$), trkB, and trkC.

The expression "extracellular domain" or "ECD" when used herein refers to any polypeptide sequence that shares a ligand binding function of the extracellular domain of a naturally occurring receptor. Ligand binding function of the extracellular domain refers to the ability of the polypeptide to bind to a ligand. Accordingly, it is not necessary to include the entire extracellular domain since smaller segments have been found to be adequate for ligand binding. The truncated extracellular domain is generally soluble. The term ECD encompasses polypeptide sequences in which the hydrophobic transmembrane sequence (and, optionally, 1-20 amino acids C-terminal and/or N-terminal to the transmembrane domain) of the mature receptor has been deleted.

The term "agonist anti-trkC antibody" refers to an antibody, which is able to bind to and activate a native sequence trkC receptor and/or downstream pathways mediated by the trkC signaling function thereby mimicking a biological activity of a native ligand of the receptor, in particular NT-3. For example, the agonist antibody may bind to the ECD domain of a trkC receptor and thereby cause dimerization of the receptor, resulting in activation of the intracellular catalytic kinase domain. Consequently, this may result in stimulation of growth and/or differentiation of cells expressing the receptor *in vitro* and/or *in vivo*. The agonist antibodies of the present invention preferably recognize an epitope that includes at least part of domain 5 (amino acid positions from about 266 to about 381) and/or domain 4 (amino acid position from about 178 to about 265) of the human trkC receptor or a corresponding epitope on a non-human, e.g. murine trkC receptor.

"Biological activity", when used in conjunction with the agonist anti-trkC antibodies of the present invention, generally refers to having an effector function in common with NT-3, the native ligand of trkC. The effector function preferably is the ability to bind and activate the trkC receptor tyrosine kinase and/or downstream pathways mediated by the trkC signaling function. Without limitation, preferred biological activities include the ability to promote the development, proliferation, maintenance and/or regeneration of damaged cells, in particular neurons *in vitro* or *in vivo*,

including peripheral (sympathetic, parasympathetic, sensory, and enteric) neurons, motorneurons, and central (brain and spinal cord) neurons, and non-neuronal cells, e.g. peripheral blood leukocytes. A particularly preferred biological activity is the ability to treat (including prevention) a neuropathy, e.g. peripheral neuropathy or other neurodegenerative disease, or repair a damaged nerve cell. The damaged neurons may be sensory, sympathetic, parasympathetic, or enteric, e.g. dorsal root ganglia neurons, motorneurons, and central neurons, e.g. neurons from the spinal cord, and the damage may be of any cause, including trauma, toxic agents, surgery, stroke, ischemia, infection, metabolic disease, nutritional deficiency, and various malignancies. Another specific biological activity is the ability to induce angiogenesis.

As used herein, "treatment" is an approach for obtaining beneficial or desired clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. "Treatment" is an intervention performed with the intention of preventing the development or altering the pathology of a disorder. Accordingly, "treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented. Specifically, the treatment may directly prevent, slow down or otherwise decrease the pathology of cellular degeneration or damage, such as the pathology of nerve cells, or may render the cells, e.g. neurons more susceptible to treatment by other therapeutic agents. In a preferred embodiment, the treatment reduces or slows down the decline and/or stimulates the restoration of the function of target neurons.

The "pathology" of a (chronic) neurodegenerative disease or acute nervous system injury includes all phenomena that affect the well being of the patient including, without limitation, neuronal disfunction, degeneration, injury and/or death.

The terms "neurodegenerative disease" and "neurodegenerative disorder" are used in the broadest sense to include all disorders the pathology of which involves neuronal degeneration and/or disfunction, including, without limitation, peripheral neuropathies; motorneuron disorders, such as amylotrophic lateral sclerosis (ALS, Lou Gehrig's disease), Bell's palsy, and various conditions involving spinal muscular atrophy or paralysis; and other human neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, epilepsy, multiple schlerosis, Huntington's chorea, Down's Syndrome, nerve deafness, and Meniere's disease.

"Peripheral neuropathy" is a neurodegenerative disorder that affects the peripheral nerves, most often manifested as one or a combination of motor, sensory, sensorimotor, or autonomic dysfunction. Peripheral neuropathies may, for example, be genetically acquired, can result from a systemic disease, or can be induced by a toxic agent, such as a neurotoxic drug, e.g. antineoplastic agent, or industrial or environmental pollutant. "Peripheral sensory neuropathy" is characterized by the degeneration of peripheral sensory neurons, which may be idiopathic, may occur, for example, as a consequence of diabetes (diabetic neuropathy), cytostatic drug therapy in cancer (e.g.

treatment with chemotherapeutic agents such as vincristine, cisplatin, methotrexate, 3'-azido-3'-deoxythymidine, or taxanes, *e.g.* paclitaxel (TAXOL®, Bristol-Myers Squibb Oncology, Princeton, NJ) and doxetaxel (TAXOTERE®, Rhône-Poulenc Rorer, Antony, France)), alcoholism, acquired immunodeficiency syndrom (AIDS), or genetic predisposition. Genetically acquired peripheral neuropathies include, for example, Refsum's disease, Krabbe's disease, Metachromatic leukodystrophy, Fabry's disease, Dejerine-Sottas syndrome, Abetalipoproteinemia, and Charcot-Marie-Tooth (CMT) Disease (also known as Proneal Muscular Atrophy or Hereditary Motor Sensory Neuropathy (HMSN)). Most types of peripheral neuropathy develop slowly, over the course of several months or years. In clinical practice such neuropathies are called chronic. Sometimes a peripheral neuropathy develops rapidly, over the course of a few days, and is referred to as acute. Peripheral neuropathy usually affects sensory and motor nerves together so as to cause a mixed sensory and motor neuropathy, but pure sensory and pure motor neuropathy are also known.

The term "toxic agent", as used in the context of the present invention, is meant to refer to a substance that, through its chemical action, injures, impairs, or inhibits the activity of a component of the nervous system. The long list of toxic agents (also referred to as "neurotoxic agents") includes, without limitation, chemotherapeutic agents, such as those listed above, alcohol, metals, industrial toxins, contaminants of food and medicines, etc.

"Mammal" for purpose of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sport or pet animals, such as dogs, horses, sheep, cats, cows, etc. Preferably, the mammal is human.

The term "trkC immunoadhesin" is used interchangeably with the expression "trkC-immunoglobulin chimera" and refers to a chimeric molecule that combines a portion of trkC (generally the extracellular domain thereof) with an immunoglobulin sequence. The immunoglobulin sequence preferably, but not necessarily, is an immunoglobulin constant domain. Chimeras constructed from a receptor sequence linked to an appropriate immunoglobulin constant domain sequence (immunoadhesins) are known in the art. Immunoadhesins reported in the literature include fusions of the T cell receptor* (Gascoigne *et al., Proc. Natl. Acad. Sci. USA*, 84: 2936-2940 [1987]); CD4* (Capon *et al., Nature* 337: 525-531 [1989]; Traunecker *et al., Nature*, 339: 68-70 [1989]; Zettmeissl *et al., DNA Cell Biol. 9*: 347-353 [1990]; Byrn *et al., Nature*, 344: 667-670 [1990]); L-selectin (homing receptor) (Watson *et al., J. Cell. Biol.*, 110:2221-2229 [1990]; Watson *et al., Nature*, 349: 164-167 [1991]); CD44* (Aruffo *et al., Cell*, 61: 1303-1313 [1990]); CD28* and B7* (Linsley *et al., J. Exp. Med.*, 173: 721-730 [1991]); CTLA-4* (Lisley *et al., J. Exp. Med.* 174: 561-569 [1991]); CD22* (Stamenkovic *et al., Cell*, 66:1133-11144 [1991]); TNF receptor (Ashkenazi *et al., Proc. Natl. Acad. Sci. USA*, 88: 10535-10539 [1991]; Lesslauer *et al., Eur. J. Immunol.*, 27:2883-2886 [1991]; Peppel *et al., J. Exp. Med.*, 174:1483-1489 [1991]); NP receptors (Bennett *et al., J. Biol. Chem.* 266:23060-23067 [1991]); and IgE receptor α* (Ridgway *et al., J. Cell. Biol.*, 115:abstr. 1448 [1991]), where the asterisk (*) indicates that the receptor is member of the immunoglobulin superfamily.

"Isolated" nucleic acid or polypeptide in the context of the present invention is a nucleic acid or polypeptide that is identified and separated from contaminant nucleic acids or polypeptides present in the animal or human source

of the nucleic acid or polypeptide. The nucleic acid or polypeptide may be labeled for diagnostic or probe purposes, using a label as described and defined further below in discussion of diagnostic assays.

In general, the term "amino acid sequence variant" refers to molecules with some differences in their amino acid sequences as compared to a reference (e.g. native sequence) polypeptide. The amino acid alterations may be substitutions, insertions, deletions or any desired combinations of such changes in a native amino acid sequence.

The terms "DNA sequence encoding", "DNA encoding" and "nucleic acid encoding" refer to the order or sequence of deoxyribonucleotides along a strand of deoxyribonucleic acid. The order of these deoxyribonucleotides determines the order of amino acids along the polypeptide chain. The DNA sequence thus codes for the amino acid sequence.

The terms "replicable expression vector" and "expression vector" refer to a piece of DNA, usually double-stranded, which may have inserted into it a piece of foreign DNA. Foreign DNA is defined as heterologous DNA, which is DNA not naturally found in the host cell. The vector is used to transport the foreign or heterologous DNA into a suitable host cell. Once in the host cell, the vector can replicate independently of the host chromosomal DNA, and several copies of the vector and its inserted (foreign) DNA may be generated. In addition, the vector contains the necessary elements that permit translating the foreign DNA into a polypeptide. Many molecules of the polypeptide encoded by the foreign DNA can thus be rapidly synthesized.

The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, a ribosome binding site, and possibly, other as yet poorly understood sequences. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancer.

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or a secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, then synthetic oligonucleotide adapters or linkers are used in accord with conventional practice.

In the context of the present invention the expressions "cell", "cell line", and "cell culture" are used interchangeably, and all such designations include progeny. Thus, the words "transformants" and "transformed (host) cells" include the primary subject cell and cultures derived therefrom without regard for the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Mutant progeny that have the same function or biological activity as screened for in the originally transformed cell are included. Where distinct designations are intended, it will be clear from the context.

An "exogenous" element is defined herein to mean nucleic acid sequence that is foreign to the cell, or homologous to the cell but in a position within the host cell nucleic acid in which the element is ordinarily not found.

"Antibodies" (Abs) and "immunoglobulins" (Igs) are glycoproteins having the same structural characteristics. While antibodies exhibit binding specificity to a specific antigen, immunoglobulins include both antibodies and other antibody-like molecules that lack antigen specificity. Polypeptides of the latter kind are, for example, produced at low levels by the lymph system and at increased levels by myelomas.

"Native antibodies" and "native immunoglobulins" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light- chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light- and heavy-chain variable domains.

The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called hypervariable regions both in the light chain and the heavy chain variable domains. The more highly conserved portions of variable domains are called the framework region (FR). The variable domains of native heavy and light chains each comprise four FRs (FR1, FR2, FR3 and FR4, respectively), largely adopting a -sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the -sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat *et al., Sequences of Proteins of Immunological Interest,* 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991), pages 647-669). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

The term "hypervariable region" when used herein refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region comprises amino acid residues from a "complementarity determining region" or "CDR" (*i.e.* residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain; Kabat *et al., Sequences of Proteins of Immunological Interest,* 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)) and/or those residues from a "hypervariable loop" (*i.e.* residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain; Chothia and Lesk *J.*

Mol. Biol. 196:901-917 (1987)). "Framework" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined.

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

"Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This region consists of a dimer of one heavy chain and one light chain variable domain in tight, non-covalent association. It is in this configuration that the three hypervariable regions of each variable domain interact to define an antigen-binding site on the surface of the V_H-V_L dimer. Collectively, the six hypervariable regions confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three hypervariable regions specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

The Fab fragment also contains the constant domain of the light chain and the first constant domain (C_H1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxyl terminus of the heavy chain C_H1 domain including one or more cysteine(s) from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa ( ) and lambda ( ), based on the amino acid sequences of their constant domains.

Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. The heavy-chain constant domains that correspond to the different classes of immunoglobulins are called , , , , and , respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

The term "antibody" herein is used in the broadest sense and specifically covers human, non-human (e.g. murine) and humanized monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), and antibody fragments so long as they exhibit the desired biological activity.

"Antibody fragments" comprise a portion of a full length antibody, generally the antigen binding or variable domain thereof. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler *et al., Nature* 256:495 (1975), or may be made by recombinant DNA methods (see, *e.g.*, U.S. Patent No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson *et al., Nature* 352:624-628 (1991) and Marks *et al., J. Mol. Biol.* 222:581-597 (1991), for example.

The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; and Morrison *et al., Proc. Natl. Acad. Sci. USA* 81:6851-6855 (1984)).

"Humanized" forms of non-human (*e.g.*, murine) antibodies are chimeric antibodies which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which hypervariable region residues of the recipient are replaced by hypervariable region residues from a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues which are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable regions correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones *et al., Nature* 321:522-525 (1986); Reichmann *et al., Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992).

"Single-chain Fv" or "sFv" antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. Generally, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the sFv to form the desired structure for antigen binding. For a review

of sFv see Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds. Springer-Verlag, New York, pp. 269-315 (1994).

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy chain variable domain (V_H) connected to a light chain variable domain (V_L) in the same polypeptide chain (V_H - V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger *et al., Proc. Natl. Acad. Sci. USA* 90:6444-6448 (1993).

The expression "linear antibodies" when used throughout this application refers to the antibodies described in Zapata *et al. Protein Eng.* 8(10):1057-1062 (1995). Briefly, these antibodies comprise a pair of tandem Fd segments (V_H-C_H1-V_H-C_H1) which form a pair of antigen binding regions. Linear antibodies can be bispecific or monospecific.

The term "epitope" is used to refer to binding sites for (monoclonal or polyclonal) antibodies on protein antigens.

Antibodies which bind to domain 5 and/or 4 within the amino acid sequence of native sequence human trkC, or to an equivalent epitope in a native sequence non-human trkC receptor, are identified by "epitope mapping." There are many methods known in the art for mapping and characterizing the location of epitopes on proteins, including solving the crystal structure of an antibody-antigen complex, competition assays, gene fragment expression assays, and synthetic peptide-based assays, as described, for example, in Chapter 11 of Harlow and Lane, Using Antibodies, a Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1999. A competition ELISA assay is specifically described in Example 1. According to the gene fragment expression assays, the open reading frame encoding the protein is fragmented either randomly or by specific genetic constructions and the reactivity of the expressed fragments of the protein with the antibody to be tested is determined. The gene fragments may, for example, be produced by PCR and then transcribed and translated into protein *in vitro*, in the presence of radioactive amino acids. The binding of the antibody to the radioactively labeled protein fragments is then determined by immunoprecipitation and gel electrophoresis. Certain epitopes can also be identified by using large libraries of random peptide sequences displayed on the surface of phage particles (phage libraries). Alternatively, a defined library of overlapping peptide fragments can be tested for binding to the test antibody in simple binding assays. The latter approach is suitable to define linear epitopes of about 5 to 15 amino acids.

An antibody binds "essentially the same epitope" as a reference antibody, when the two antibodies recognize identical or sterically overlapping epitopes. The most widely used and rapid methods for determining whether two epitopes bind to identical or sterically overlapping epitopes are competition assays, which can be configured in all number of different formats, using either labeled antigen or labeled antibody. Usually, the antigen is immobilized on a 96-well plate, and the ability of unlabeled antibodies to block the binding of labeled antibodies is measured using radioactive or enzyme labels. A competition ELISA assay is disclosed in Example 1.

The term amino acid or amino acid residue, as used herein, refers to naturally occurring L amino acids or to D amino acids as described further below with respect to variants. The commonly used one- and three-letter abbreviations for amino acids are used herein (Bruce Alberts et al., Molecular Biology of the Cell, Garland Publishing, Inc., New York (3d ed. 1994)).

Hybridization is preferably performed under "stringent conditions" which means (1) employing low ionic strength and high temperature for washing, for example, 0.015 sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50 C, or (2) employing during hybridization a denaturing agent, such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42 C. Another example is use of 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6/8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 g/ml), 0.1% SDS, and 10% dextran sulfate at 42 C, with washes at 42 C in 0.2 x SSC and 0.1% SDS.

B. Methods for carrying out the invention

The present invention concerns agonist human and non-human monoclonal antibodies (including humanized forms of the latter), which mimick certain biological properties of NT-3, the native ligand of the trkC receptor. General techniques for the production of murine and human anti-trkC antibodies are well known in the art and are described hereinbelow. Further details, including the selection of agonist antibodies, are provided in Example 1.

1. Antibody preparation

(i) Polyclonal antibodies

Methods of preparing polyclonal antibodies are known in the art. Polyclonal antibodies can be raised in a mammal, for example, by one or more injections of an immunizing agent and, if desired, an adjuvant. Typically, the immunizing agent and/or adjuvant will be injected in the mammal by multiple subcutaneous or intraperitoneal injections. It may be useful to conjugate the immunizing agent to a protein known to be immunogenic in the mammal being immunized, such as serum albumin, or soybean trypsin inhibitor. Examples of adjuvants which may be employed include Freund's complete adjuvant and MPL-TDM.

(ii) Monoclonal antibodies

Monoclonal antibodies may be made using the hybridoma method first described by Kohler *et al., Nature,* 256:495 (1975), or may be made by recombinant DNA methods (U.S. Patent No. 4,816,567).

In the hybridoma method, a mouse or other appropriate host animal, such as a hamster or macaque monkey, is immunized as hereinabove described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Alternatively, lymphocytes may be immunized *in vitro*. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103, (Academic Press, 1986)).

The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For

example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), conditions under which the growth of HGPRT-deficient cells is prevented.

Preferred myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these, preferred myeloma cell lines are murine myeloma lines, such as those derived from MOP-21 and M.C.-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, California USA, and SP-2 or X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Maryland USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, *J. Immunol.*, 133:3001 (1984); Brodeur *et al., Monoclonal Antibody Production Techniques and Applications*, pp. 51-63, Marcel Dekker, Inc., New York, (1987)).

Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA).

The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson *et al., Anal. Biochem.*, 107:220 (1980).

After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the cells may be subcloned by limiting dilution procedures and grown by standard methods (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103 (Academic Press, 1986)). Suitable culture media for this purpose include, for example, DMEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown *in vivo* as ascites tumors in an animal.

The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the monoclonal antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also may be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences, Morrison, *et al.*, Proc. Nat. Acad. Sci. 81, 6851 (1984), or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. In that

manner, "chimeric" or "hybrid" antibodies are prepared that have the binding specificity of an anti-trk monoclonal antibody herein.

Typically such non-immunoglobulin polypeptides are substituted for the constant domains of an antibody of the invention, or they are substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for an trk receptor and another antigen-combining site having specificity for a different antigen.

Chimeric or hybrid antibodies also may be prepared in vitro using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate.

Recombinant production of antibodies will be described in more detail below.

(iii) Humanized antibodies

Generally, a humanized antibody has one or more amino acid residues introduced into it from a non-human source. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers [Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature, 332:323-327 (1988); Verhoeyen et al., Science, 239:1534-1536 (1988)], by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody.

Accordingly, such "humanized" antibodies are chimeric antibodies (Cabilly, supra), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

It is important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three dimensional models of the parental and humanized sequences. Three dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e. the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the consensus and import sequence so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding. For further details see U.S. application Serial No. 07/934,373 filed 21 August 192, which is a continuation-in-part of application Serial No. 07/715,272 filed 14 June 1991.

(iv) Human antibodies

Human monoclonal antibodies can be made by the hybridoma method. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described, for example, by Kozbor, J. Immunol. 133, 3001 (1984), and Brodeur, *et al.*, Monoclonal Antibody Production Techniques and Applications, pp.51-63 (Marcel Dekker, Inc., New York, 1987).

It is now possible to produce transgenic animals (e.g. mice) that are capable, upon immunization, of producing a repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, e.g. Jakobovits *et al.*, Proc. Natl. Acad. Sci. USA 90, 2551-255 (1993); Jakobovits *et al.*, Nature 362, 255-258 (1993).

Mendez *et al.* (*Nature Genetics* 15: 146-156 [1997]) have further improved the technology and have generated a line of transgenic mice designated as "Xenomouse II" that, when challenged with an antigen, generates high affinity fully human antibodies. This was achieved by germ-line integration of megabase human heavy chain and light chain loci into mice with deletion into endogenous J_H segment as described above. The Xenomouse II harbors 1,020 kb of human heavy chain locus containing approximately 66 V_H genes, complete D_H and J_H regions and three different constant regions (μ, δ and χ), and also harbors 800 kb of human κ locus containing 32 Vκ genes, Jκ segments and Cκ genes. The antibodies produced in these mice closely resemble that seen in humans in all respects, including gene rearrangement, assembly, and repertoire. The human antibodies are preferentially expressed over endogenous antibodies due to deletion in endogenous J_H segment that prevents gene rearrangement in the murine locus.

Alternatively, the phage display technology (McCafferty *et al.*, Nature 348, 552-553 [1990]) can be used to produce human antibodies and antibody fragments *in vitro*, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors. According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimicks some of the properties of the B-cell. Phage display can be performed in a variety of formats; for their review see, e.g. Johnson, Kevin S. and Chiswell, David J., Current Opinion in Structural Biology 3, 564-571 (1993). Several sources of V-gene segments can be used for phage display. Clackson *et al.*, Nature 352, 624-628 (1991) isolated a diverse array of anti-oxazolone antibodies from a small random combinatorial library of V genes derived from the spleens of immunized mice. A repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Marks *et al.*, J. Mol. Biol. 222, 581-597 (1991), or Griffith *et al.*, EMBO J. 12, 725-734 (1993). In a natural immune response, antibody genes accumulate

mutations at a high rate (somatic hypermutation). Some of the changes introduced will confer higher affinity, and B cells displaying high-affinity surface immunoglobulin are preferentially replicated and differentiated during subsequent antigen challenge. This natural process can be mimicked by employing the technique known as "chain shuffling" (Marks *et al.*, Bio/Technol. 10, 779-783 (1992)). In this method, the affinity of "primary" human antibodies obtained by phage display can be improved by sequentially replacing the heavy and light chain V region genes with repertoires of naturally occurring variants (repertoires) of V domain genes obtained from unimmunized donors. This techniques allows the production of antibodies and antibody fragments with affinities in the nM range. A strategy for making very large phage antibody repertoires (also known as "the mother-of-all libraries") has been described by Waterhouse *et al.*, Nucl. Acids Res. 21, 2265-2266 (1993), and the isolation of a high affinity human antibody directly from such large phage library is reported by Griffith *et al.*, EMBO J. (1994), in press. Gene shuffling can also be used to derive human antibodies from rodent antibodies, where the human antibody has similar affinities and specificities to the starting rodent antibody. According to this method, which is also referred to as "epitope imprinting", the heavy or light chain V domain gene of rodent antibodies obtained by phage display technique is replaced with a repertoire of human V domain genes, creating rodent-human chimeras. Selection on antigen results in isolation of human variable capable of restoring a functional antigen-binding site, i.e. the epitope governs (imprints) the choice of partner. When the process is repeated in order to replace the remaining rodent V domain, a human antibody is obtained (see PCT patent application WO 93/06213, published 1 April 1993). Unlike traditional humanization of rodent antibodies by CDR grafting, this technique provides completely human antibodies, which have no framework or CDR residues of rodent origin.

(v) Bispecific antibodies

Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for the trkC receptor to provide an agonist antibody, the other one is for any other antigen, and preferably for another receptor or receptor subunit. For example, bispecific antibodies specifically binding a trkC receptor and a neurotrophin, or a trkC receptor and another trk receptor are within the scope of the present invention.

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the coexpression of two immunoglobulin heavy chain-light chain pairs, where the two heavy chains have different specificities (Millstein and Cuello, Nature 305, 537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in PCT application publication No. WO 93/08829 (published 13 May 1993), and in Traunecker *et al.*, EMBO 10, 3655-3659 (1991).

According to a different and more preferred approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion

preferably is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, CH2 and CH3 regions. It is preferred to have the first heavy chain constant region (CH1) containing the site necessary for light chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are cotransfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance. In a preferred embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in PCT Publication No. WO 94/04690, published on March 3, 1994.

For further details of generating bispecific antibodies see, for example, Suresh *et al.*, Methods in Enzymology 121, 210 (1986).

(vi) Heteroconjugate antibodies

Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Patent No. 4,676,980), and for treatment of HIV infection (PCT application publication Nos. WO 91/00360 and WO 92/200373; EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. Patent No. 4,676,980, along with a number of cross-linking techniques.

(vii)Antibody fragments

In certain embodiments, the anti-trkC antibody (including murine, human and humanized antibodies, and antibody variants) is an antibody fragment. Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, *e.g.*, Morimoto *et al.*, *J. Biochem. Biophys. Methods* 24:107-117 (1992) and Brennan *et al.*, *Science* 229:81 (1985)). However, these fragments can now be produced directly by recombinant host cells. For example, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form $F(ab')_2$ fragments (Carter *et al.*, *Bio/Technology* 10:163-167 (1992)). In another embodiment, the $F(ab')_2$ is formed using the leucine zipper GCN4 to promote assembly of the $F(ab')_2$ molecule. According to another approach, Fv, Fab or $F(ab')_2$ fragments can be isolated directly from recombinant host cell culture. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner.

(viii) Amino acid sequence variants of antibodies

Amino acid sequence variants of the anti-trkC antibodies are prepared by introducing appropriate nucleotide changes into the anti-trkC antibody DNA, or by peptide synthesis. Such variants include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the anti-trkC antibodies of the examples herein. Any combination of deletion, insertion, and substitution is made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes also may alter post-translational processes of the humanized or variant anti-trkC antibody, such as changing the number or position of glycosylation sites.

A useful method for identification of certain residues or regions of the anti-trkC antibody that are preferred locations for mutagenesis is called "alanine scanning mutagenesis," as described by Cunningham and Wells *Science*, 244:1081-1085 (1989). Here, a residue or group of target residues are identified (*e.g.*, charged residues such as arg, asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with trkC antigen. Those amino acid locations demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at, or for, the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation *per se* need not be predetermined. For example, to analyze the performance of a mutation at a given site, ala scanning or random mutagenesis is conducted at the target codon or region and the expressed anti-trkC antibody variants are screened for the desired activity.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an anti-trkC antibody with an N-terminal methionyl residue or the antibody fused to an epitope tag. Other insertional variants of the anti-trkC antibody molecule include the fusion to the N- or C-terminus of the anti-trkC antibody of an enzyme or a polypeptide which increases the serum half-life of the antibody (see below).

Another type of variant is an amino acid substitution variant. These variants have at least one amino acid residue in the anti-trkC antibody molecule removed and a different residue inserted in its place. The sites of greatest interest for substitutional mutagenesis include the hypervariable regions, but FR alterations are also contemplated. Conservative substitutions are shown in Table 1 under the heading of "preferred substitutions". If such substitutions result in a change in biological activity, then more substantial changes, denominated "exemplary substitutions" in Table 1, or as further described below in reference to amino acid classes, may be introduced and the products screened.

Table 1

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	val; leu; ile	val
Arg (R)	lys; gln; asn	lys
Asn (N)	gln; his; asp, lys; arg	gln
Asp (D)	glu; asn	glu
Cys (C)	ser; ala	ser
Gln (Q)	asn; glu	asn
Glu (E)	asp; gln	asp
Gly (G)	ala	ala
His (H)	asn; gln; lys; arg	arg
Ile (I)	leu; val; met; ala; phe; norleucine	leu
Leu (L)	norleucine; ile; val; met; ala; phe	ile
Lys (K)	arg; gln; asn	arg
Met (M)	leu; phe; ile	leu
Phe (F)	leu; val; ile; ala; tyr	tyr
Pro (P)	ala	ala
Ser (S)	thr	thr
Thr (T)	ser	ser
Trp (W)	tyr; phe	tyr
Tyr (Y)	trp; phe; thr; ser	phe

Val (V)	ile; leu; met; phe; ala; norleucine	leu

Substantial modifications in the biological properties of the antibody are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

(1) hydrophobic: norleucine, met, ala, val, leu, ile;

(2) neutral hydrophilic: cys, ser, thr;

(3) acidic: asp, glu;

(4) basic: asn, gln, his, lys, arg;

(5) residues that influence chain orientation: gly, pro; and

(6) aromatic: trp, tyr, phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

Any cysteine residue not involved in maintaining the proper conformation of the humanized or variant anti-trkC antibody also may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking. Conversely, cysteine bond(s) may be added to the antibody to improve its stability (particularly where the antibody is an antibody fragment such as an Fv fragment).

A particularly preferred type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (*e.g.* a humanized or human antibody). Generally, the resulting variant(s) selected for further development will have improved biological properties relative to the parent antibody from which they are generated. A convenient way for generating such substitutional variants is affinity maturation using phage display. Briefly, several hypervariable region sites (*e.g.* 6-7 sites) are mutated to generate all possible amino substitutions at each site. The antibody variants thus generated are displayed in a monovalent fashion from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed variants are then screened for their biological activity (*e.g.* binding affinity) as herein disclosed. In order to identify candidate hypervariable region sites for modification, alanine scanning mutagenesis can be performed to identify hypervariable region residues contributing significantly to antigen binding. Alternatively, or in addition, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the antibody and human trkC. Such contact residues and neighboring residues are candidates for substitution according to the techniques elaborated herein. Once such variants are generated, the panel of variants is subjected to screening as described herein and antibodies with superior properties in one or more relevant assays may be selected for further development.

(ix) Glycosylation variants of antibodies

Antibodies are glycosylated at conserved positions in their constant regions (Jefferis and Lund, Chem. Immunol. 65:111-128 [1997]; Wright and Morrison, TibTECH 15:26-32 [1997]). The oligosaccharide side chains of the immunoglobulins affect the protein's function (Boyd *et al.*, Mol. Immunol. 32:1311-1318 [1996]; Wittwe and Howard, Biochem. 29:4175-4180 [1990]), and the intramolecular interaction between portions of the glycoprotein which can affect the conformation and presented three-dimensional surface of the glycoprotein (Hefferis and Lund, *supra*; Wyss and Wagner, Current Opin. Biotech. 7:409-416 [1996]). Oligosaccharides may also serve to target a given glycoprotein to certain molecules based upon specific recognition structures. For example, it has been reported that in agalactosylated IgG, the oligosaccharide moiety 'flips' out of the inter-CH2 space and terminal N-acetylglucosamine residues become available to bind mannose binding protein (Malhotra *et al.*, Nature Med. 1:237-243 [1995]). Removal by glycopeptidase of the oligosaccharides from CAMPATH-1H (a recombinant humanized murine monoclonal IgG1 antibody which recognizes the CDw52 antigen of human lymphocytes) produced in Chinese Hamster Ovary (CHO) cells resulted in a complete reduction in complement mediated lysis (CMCL) (Boyd *et al.*, Mol. Immunol. 32:1311-1318 [1996]), while selective removal of sialic acid residues using neuraminidase resulted in no loss of DMCL. Glycosylation of antibodies has also been reported to affect antibody-dependent cellular cytotoxicity (ADCC). In particular, CHO cells with tetracycline-regulated expression of β(1,4)-N-acetylglucosaminyltransferase III (GnTIII), a glycosyltransferase catalyzing formation of bisecting GlcNAc, was reported to have improved ADCC activity (Umana *et al.*, Mature Biotech. 17:176-180 [1999]).

Glycosylation variants of antibodies are variants in which the glycosylation pattern of an antibody is altered. By altering is meant deleting one or more carbohydrate moieties found in the antibody, adding one or more carbohydrate moieties to the antibody, changing the composition of glycosylation (glycosylation pattern), the extent of glycosylation, etc. Glycosylation variants may, for example, be prepared by removing, changing and/or adding one or more glycosylation sites in the nucleic acid sequence encoding the antibody.

Glycosylation of antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

Addition of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation sites).

Nucleic acid molecules encoding amino acid sequence variants of the anti-trkC antibody are prepared by a variety of methods known in the art. These methods include, but are not limited to, isolation from a natural source (in

the case of naturally occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of the anti-trkC antibody.

The glycosylation (including glycosylation pattern) of antibodies may also be altered without altering the underlying nucleotide sequence. Glycosylation largely depends on the host cell used to express the antibody. Since the cell type used for expression of recombinant glycoproteins, e.g. antibodies, as potential therapeutics is rarely the native cell, significant variations in the glycosylation pattern of the antibodies can be expected (see, e.g. Hse *et al.*, J. Biol. Chem. 272:9062-9070 (1997)). In addition to the choice of host cells, factors which affect glycosylation during recombinant production of antibodies include growth mode, media formulation, culture density, oxygenation, pH, purification schemes and the like. Various methods have been proposed to alter the glycosylation pattern achieved in a particular host organism including introducing or overexpressing certain enzymes involved in oligosaccharide production (U. S. Patent Nos. 5,047,335; 5,510,261 and 5,278,299). Glycosylation, or certain types of glycosylation, can be enzymatically removed from the glycoprotein, for example using endoglycosidase H (Endo H). In addition, the recombinant host cell can be genetically engineered, e.g. make defective in processing certain types of polysaccharides. These and similar techniques are well known in the art.

The glycosylation structure of antibodies can be readily analyzed by conventional techniques of carbohydrate analysis, including lectin chromatography, NMR, Mass spectrometry, HPLC, GPC, monosaccharide compositional analysis, sequential enzymatic digestion, and HPAEC-PAD, which uses high pH anion exchange chromatography to separate oligosaccharides based on charge. Methods for releasing oligosaccharides for analytical purposes are also known, and include, without limitation, enzymatic treatment (commonly performed using peptide-N-glycosidase F/endo-β-galactosidase), elimination using harsh alkaline environment to release mainly O-linked structures, and chemical methods using anhydrous hydrazine to release both N- and O-linked oligosaccharides.

(x) Other modifications of antibodies

The anti-trkC antibodies disclosed herein may also be formulated as immunoliposomes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein *et al., Proc. Natl. Acad. Sci. USA* 82:3688 (1985); Hwang *et al., Proc. Natl Acad. Sci. USA* 77:4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present invention can be conjugated to the liposomes as described in Martin *et al., J. Biol. Chem.* 257:286-288 (1982) via a disulfide interchange reaction. A chemotherapeutic agent (such as Doxorubicin) is optionally contained within the liposome. See Gabizon *et al., J. National Cancer Inst.*81(19):1484 (1989).

The antibody of the present invention may also be used in ADEPT by conjugating the antibody to a prodrug-activating enzyme which converts a prodrug (*e.g.*, a peptidyl chemotherapeutic agent, see WO81/01145) to an active anti-cancer drug. See, for example, WO 88/07378 and U.S. Patent No. 4,975,278.

The enzyme component of the immunoconjugate useful for ADEPT includes any enzyme capable of acting on a prodrug in such a way so as to covert it into its more active, cytotoxic form.

Enzymes that are useful in the method of this invention include, but are not limited to, alkaline phosphatase useful for converting phosphate-containing prodrugs into free drugs; arylsulfatase useful for converting sulfate-containing prodrugs into free drugs; cytosine deaminase useful for converting non-toxic 5-fluorocytosine into the anti-cancer drug, 5-fluorouracil; proteases, such as serratia protease, thermolysin, subtilisin, carboxypeptidases and cathepsins (such as cathepsins B and L), that are useful for converting peptide-containing prodrugs into free drugs; D-alanylcarboxypeptidases, useful for converting prodrugs that contain D-amino acid substituents; carbohydrate-cleaving enzymes such as -galactosidase and neuraminidase useful for converting glycosylated prodrugs into free drugs; -lactamase useful for converting drugs derivatized with -lactams into free drugs; and penicillin amidases, such as penicillin V amidase or penicillin G amidase, useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups, respectively, into free drugs. Alternatively, antibodies with enzymatic activity, also known in the art as "abzymes", can be used to convert the prodrugs of the invention into free active drugs (see, *e.g.*, Massay, *Nature* 328:457-458 (1987)). Antibody-abzyme conjugates can be prepared as described herein for delivery of the abzyme to a tumor cell population.

The enzymes of this invention can be covalently bound to the anti-trkC antibodies by techniques well known in the art such as the use of the heterobifunctional crosslinking reagents discussed above. Alternatively, fusion proteins comprising at least the antigen binding region of an antibody of the invention linked to at least a functionally active portion of an enzyme of the invention can be constructed using recombinant DNA techniques well known in the art (see, *e.g.*, Neuberger *et al.*, *Nature* 312:604-608 (1984)).

In certain embodiments of the invention, it may be desirable to use an antibody fragment, rather than an intact antibody. In this case, it may be desirable to modify the antibody fragment in order to increase its serum half-life. This may be achieved, for example, by incorporation of a salvage receptor binding epitope into the antibody fragment (*e.g.*, by mutation of the appropriate region in the antibody fragment or by incorporating the epitope into a peptide tag that is then fused to the antibody fragment at either end or in the middle, *e.g.*, by DNA or peptide synthesis). See WO96/32478 published October 17, 1996.

The salvage receptor binding epitope generally constitutes a region wherein any one or more amino acid residues from one or two loops of a Fc domain are transferred to an analogous position of the antibody fragment. Even more preferably, three or more residues from one or two loops of the Fc domain are transferred. Still more preferred, the epitope is taken from the CH2 domain of the Fc region (*e.g.*, of an IgG) and transferred to the CH1, CH3, or V_H region, or more than one such region, of the antibody. Alternatively, the epitope is taken from the CH2 domain of the Fc region and transferred to the C_L region or V_L region, or both, of the antibody fragment.

Covalent modifications of the humanized or variant anti-trkC antibody (including glycosylation variants) are also included within the scope of this invention. They may be made by chemical synthesis or by enzymatic or chemical cleavage of the antibody, if applicable. Other types of covalent modifications of the antibody are introduced into the molecule by reacting targeted amino acid residues of the antibody with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues. Exemplary covalent modifications of polypeptides are described in US Patent 5,534,615, specifically incorporated herein by reference. A preferred type of covalent modification of the antibody comprises linking the antibody to one of a variety of nonproteinaceous polymers, *e.g.*, polyethylene glycol, polypropylene glycol, or polyoxyalkylenes, in the manner set forth in U.S. Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337.

2. Vectors, Host Cells and Recombinant Methods

The invention also provides isolated nucleic acid encoding the non-human, e.g. murine and human anti-trkC antibodies of the present invention (including the humanized versions of the non-human antibodies), vectors and host cells comprising the nucleic acid, and recombinant techniques for the production of the antibodies.

For recombinant production of an antibody, the nucleic acid encoding it may be isolated and inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. In another embodiment, the antibody may be produced by homologous recombination, *e.g.* as described in US Patent 5,204,244, specifically incorporated herein by reference. DNA encoding the monoclonal antibody is readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody). Many vectors are available. The vector components generally include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence, *e.g.*, as described in US Patent 5,534,615 issued July 9, 1996 and specifically incorporated herein by reference.

Suitable host cells for cloning or expressing the DNA in the vectors herein are the prokaryote, yeast, or higher eukaryote cells described above. Suitable prokaryotes for this purpose include eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as *Escherichia*, *e.g.*, *E. coli*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Proteus*, *Salmonella*, *e.g.*, *Salmonella typhimurium*, *Serratia*, *e.g.*, *Serratia marcescans*, and *Shigella*, as well as *Bacilli* such as *B. subtilis* and *B. licheniformis* (*e.g.*, *B. licheniformis* 41P disclosed in DD 266,710 published 12 April 1989), *Pseudomonas* such as *P. aeruginosa*, and *Streptomyces*. One preferred *E. coli* cloning host is *E. coli* 294 (ATCC 31,446), although other strains such as *E. coli* B, *E. coli* X1776 (ATCC 31,537), and *E. coli* W3110 (ATCC 27,325) are suitable. These examples are illustrative rather than limiting.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for anti-trkC antibody-encoding vectors. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *Schizosaccharomyces pombe*; *Kluyveromyces* hosts such as, *e.g.*, *K. lactis*, *K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC 16,045), *K. wickeramii* (ATCC 24,178), *K. waltii*

[ATCC 56,500), *K. drosophilarum* (ATCC 36,906), *K . thermotolerans*, and *K. marxianus; yarrowia* (EP 402,226); *Pichia pastoris* (EP 183,070); *Candida; Trichoderma reesia* (EP 244,234); *Neurospora crassa; Schwanniomyces* such as *Schwanniomyces occidentalis*; and filamentous fungi such as, *e.g., Neurospora, Penicillium, Tolypocladium*, and *Aspergillus* hosts such as *A. nidulans* and *A. niger*.

Suitable host cells for the expression of glycosylated anti-trkC antibody are derived from multicellular organisms. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda* (caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosophila melanogaster* (fruitfly), and *Bombyx mori* have been identified. A variety of viral strains for transfection are publicly available, *e.g.*, the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of *Spodoptera frugiperda* cells. Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, and tobacco can also be utilized as hosts.

However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al., J. Gen Virol.* 36:59 [1977]); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub *et al., Proc. Natl. Acad. Sci. USA* 77:4216 [1980]); mouse sertoli cells (TM4, Mather, *Biol. Reprod.* 23:243-251 [1980]); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HeLa, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather *et al., Annals N.Y. Acad. Sci.* 383:44-68 [1982]); MRC 5 cells; and FS4 cells.

Host cells are transformed with the above-described expression or cloning vectors for anti-trkC antibody production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

The host cells used to produce the anti-trkC antibody of this invention may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ((MEM) (Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium (DMEM) (Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham *et al., Meth. Enz.* 58:44 (1979), Barnes *et al., Anal. Biochem.*102:255 (1980), U.S. Pat. Nos. 4,767,704; 4,657,866; 4,927,762; 4,560,655; or 5,122,469; WO 90/03430; WO 87/00195; or U.S. Patent Re. 30,985 may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleotides (such as adenosine and thymidine), antibiotics (such as GENTAMYCIN™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any

other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

When using recombinant techniques, the antibody can be produced intracellularly, in the periplasmic space, or directly secreted into the medium. If the antibody is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, is removed, for example, by centrifugation or ultrafiltration. Carter *et al.*, *Bio/Technology* 10:163-167 (1992) describe a procedure for isolating antibodies which are secreted to the periplasmic space of *E. coli*. Briefly, cell paste is thawed in the presence of sodium acetate (pH 3.5), EDTA, and phenylmethylsulfonylfluoride (PMSF) over about 30 min. Cell debris can be removed by centrifugation. Where the antibody is secreted into the medium, supernatants from such expression systems are generally first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. A protease inhibitor such as PMSF may be included in any of the foregoing steps to inhibit proteolysis and antibiotics may be included to prevent the growth of adventitious contaminants.

The antibody composition prepared from the cells can be purified using, for example, hydroxylapatite chromatography, gel electrophoresis, dialysis, and affinity chromatography, with affinity chromatography being the preferred purification technique. The suitability of protein A as an affinity ligand depends on the species and isotype of any immunoglobulin Fc domain that is present in the antibody. Protein A can be used to purify antibodies that are based on human 1, 2, or 4 heavy chains (Lindmark *et al., J. Immunol. Meth.* 62:1-13 (1983)). Protein G is recommended for all mouse isotypes and for human 3 (Guss *et al., EMBO J.* 5:15671575 (1986)). The matrix to which the affinity ligand is attached is most often agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrenedivinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Where the antibody comprises a C_H3 domain, the Bakerbond ABX™ resin (J. T. Baker, Phillipsburg, NJ) is useful for purification. Other techniques for protein purification such as fractionation on an ion-exchange column, ethanol precipitation, Reverse Phase HPLC, chromatography on silica, chromatography on heparin SEPHAROSE™ chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also available depending on the antibody to be recovered.

Following any preliminary purification step(s), the mixture comprising the antibody of interest and contaminants may be subjected to low pH hydrophobic interaction chromatography using an elution buffer at a pH between about 2.5-4.5, preferably performed at low salt concentrations (*e.g.*, from about 0-0.25M salt).

3. Identification of Agonist anti-trkC Antibodies

Agonist antibodies may be identified, for example, using the kinase receptor activation (KIRA) assay described in U. S. Patent Nos. 5,766,863 and 5,891,650. This ELISA-type assay is suitable for qualitative or quantitative measurement of kinase activation by measuring the autophosphorylation of the kinase domain of a receptor protein tyrosine kinase (rPTK, e.g. trk receptor), as well as for identification and characterization of potential

agonist or antagonists of a selected rPTK. The first stage of the assay involves phosphorylation of the kinase domain of a kinase receptor, in the present case a trkC receptor, wherein the receptor is present in the cell membrane of a eukaryotic cell. The receptor may be an endogenous receptor or nucleic acid encoding the receptor, or a receptor construct, may be transformed into the cell. Typically, a first solid phase (*e.g.*, a well of a first assay plate) is coated with a substantially homogeneous population of such cells (usually a mammalian cell line) so that the cells adhere to the solid phase. Ditan, the cells are adherent and thereby adhere naturally to the first solid phase. If a "receptor construct" is used, it usually comprises a fusion of a kinase receptor and a flag polypeptide. The flag polypeptide is recognized by the capture agent, often a capture antibody, in the ELISA part of the assay. An analyte, such as a candidate agonist, is then added to the wells having the adherent cells, such that the tyrosine kinase receptor (e.g. trkC receptor) is exposed to (or contacted with) the analyte. This assay enables identification of agonist ligands for the tyrosine kinase receptor of interest (e.g. trkC). It is also possible to use this assay to detect antagonists of a tyrosine kinase receptor. In order to detect the presence of an antagonist ligand which blocks binding of an agonist to the receptor, the adhering cells are exposed to the suspected antagonist ligand first, and then to the agonist ligand, so that competitive inhibition of receptor binding and activation can be measured. Also, the assay can identify an antagonist which binds to the agonist ligand and thereby reduces or eliminates its ability to bind to, and activate, the rPTK. To detect such an antagonist, the suspected antagonist and the agonist for the rPTK are incubated together and the adhering cells are then exposed to this mixture of ligands. Following exposure to the analyte, the adhering cells are solubilized using a lysis buffer (which has a solubilizing detergent therein) and gentle agitation, thereby releasing cell lysate which can be subjected to the ELISA part of the assay directly, without the need for concentration or clarification of the cell lysate.

The cell lysate thus prepared is then ready to be subjected to the ELISA stage of the assay. As a first step in the ELISA stage, a second solid phase (usually a well of an ELISA microtiter plate) is coated with a capture agent (often a capture antibody) which binds specifically to the tyrosine kinase receptor, or, in the case of a receptor construct, to the flag polypeptide. Coating of the second solid phase is carried out so that the capture agent adheres to the second solid phase. The capture agent is generally a monoclonal antibody, but, as is described in the examples herein, polyclonal antibodies may also be used. The cell lysate obtained is then exposed to, or contacted with, the adhering capture agent so that the receptor or receptor construct adheres to (or is captured in) the second solid phase. A washing step is then carried out, so as to remove unbound cell lysate, leaving the captured receptor or receptor construct. The adhering or captured receptor or receptor construct is then exposed to, or contacted with, an anti-phosphotyrosine antibody which identifies phosphorylated tyrosine residues in the tyrosine kinase receptor. In the preferred embodiment, the anti-phosphotyrosine antibody is conjugated (directly or indirectly) to an enzyme which catalyses a color change of a non-radioactive color reagent. Accordingly, phosphorylation of the receptor can be measured by a subsequent color change of the reagent. The enzyme can be bound to the anti-phosphotyrosine antibody directly, or a conjugating molecule (*e.g.*, biotin) can be conjugated to the anti-phosphotyrosine antibody and the enzyme can be subsequently bound to the anti-phosphotyrosine antibody via the conjugating molecule. Finally,

binding of the anti-phosphotyrosine antibody to the captured receptor or receptor construct is measured, *e.g.*, by a color change in the color reagent.

Following initial identification, the agonist activity can be further confirmed and refined by bioassays, known to test the targeted biological activities. For example, the ability of anti-trkC monoclonal antibodies to mimic the activity of NT-3 can be tested in the PC12 neurite outgrowth assay as described in Example 1, and confirmed in known animal models of neurodegenerative diseases, such as the experimental animal models of cisplatin- and pyridoxine-induced neuropathies described in Example 2.

3. Therapeutic and Diagnostic Uses of Agonist anti-TrkC Antibodies

The anti-trkC agonist antibodies of the present invention are believed to be useful in the treatment (including prevention) of disorders the pathology of which involves cellular degeneration or disfunction. In particular, the anti-trkC agonist antibodies are promising candidates for the treatment of various (chronic) neurodegenerative disorders and acute nerve cell injuries. Such neurodegenerative disorders include, without limitation, peripheral neuropathies; motorneuron disorders, such as amylotrophic lateral sclerosis (ALS, Lou Gehrig's disease), Bell's palsy, and various conditions involving spinal muscular atrophy or paralysis; and other human neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, epilepsy, multiple sclerosis, Huntington's chorea, Down's Syndrome, nerve deafness, and Meniere's disease, and acute nerve cell injuries, for example due to trauma or spinal cord injury.

The anti-trkC antibodies of the present invention are believed to be particularly suited for the treatment of peripheral neuropathy, a neurodegenerative disorder that affects the peripheral nerves, most often manifested as one or a combination of motor, sensory, sensorimotor, or autonomic dysfunction. Peripheral neuropathies may, for example, be genetically acquired, can result from a systemic disease, can be induced by a toxic agent, such as a neurotoxic drug, e.g. antineoplastic agent, or industrial or environmental pollutant, or can be idiopathic. Thus, peripheral sensory neuropathy is characterized by the degeneration, decrease or failure of function of peripheral sensory neurons, which may occur, for example, as a consequence of diabetes (diabetic neuropathy), cytostatic drug therapy in cancer (e.g. treatment with chemotherapeutic agents such as vincristine, cisplatin, methotrexate, or 3'-azido-3'-deoxythymidine), alcoholism, acquired immunodeficiency syndrome (AIDS), or genetic predisposition. Genetically acquired peripheral neuropathies include, for example, Refsum's disease, Krabbe's disease, Metachromatic leukodystrophy, Fabry's disease, Dejerine-Sottas syndrome, Abetalipoproteinemia, and Charcot-Marie-Tooth (CMT) Disease (also known as Proneal Muscular Atrophy or Hereditary Motor Sensory Neuropathy (HMSN)).

Based on the demonstrated ability of NT-3, the native ligand of the trkC receptor, to promote proliferation of peripheral blood leukocytes, the anti-trkC agonist antibodies of the present invention may be used also as therapeutic agents for the treatment of neutropenia, various infections, and tumors. Since the expression of trkC is not limited to neurons, anti-trkC agonist antibodies are expected to find utility in the prevention or treatment of disorders characterized by cellular degeneration in general, without restriction to neural cells.

The anti-trkC antibodies of the present invention may also be used to induce angiogenesis, or treat pathological conditions/diseases in which the induction of angiogenesis is desirable. Such pathological conditions include, for example, cardiac ischemia regardless of the underlying pathology, including cerebrovascular disorders caused by insufficient cerebral circulation. Angiogenesis may also be desirable in the treatment of wounds, including ulcers, diabetic complications of sickle cell disease, and post surgical wounds.

The anti-trkC antibodies of the present invention may also be useful in the diagnosis of diseases involving cellular degeneration, in particular the neurodegenerative diseases listed above.

For diagnostic applications, the antibody typically will be labeled with a detectable moiety. Numerous labels are available which can be generally grouped into the following categories:

(a) Radioisotopes, such as ^{35}S, ^{14}C, ^{125}I, ^{3}H, and ^{131}I. The antibody can be labeled with the radioisotope using the techniques described in *Current Protocols in Immunology*, Volumes 1 and 2, Coligen *et al.*, Ed. Wiley-Interscience, New York, New York, Pubs. (1991) for example and radioactivity can be measured using scintillation counting.

(b) Fluorescent labels such as rare earth chelates (europium chelates) or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, Lissamine, phycoerythrin and Texas Red are available. The fluorescent labels can be conjugated to the antibody using the techniques disclosed in *Current Protocols in Immunology, supra*, for example. Fluorescence can be quantified using a fluorimeter.

(c) Various enzyme-substrate labels are available and U.S. Patent No. 4,275,149 provides a review of some of these. The enzyme generally catalyzes a chemical alteration of the chromogenic substrate which can be measured using various techniques. For example, the enzyme may catalyze a color change in a substrate, which can be measured spectrophotometrically. Alternatively, the enzyme may alter the fluorescence or chemiluminescence of the substrate. Techniques for quantifying a change in fluorescence are described above. The chemiluminescent substrate becomes electronically excited by a chemical reaction and may then emit light which can be measured (using a chemiluminometer, for example) or donates energy to a fluorescent acceptor. Examples of enzymatic labels include luciferases (*e.g.*, firefly luciferase and bacterial luciferase; U.S. Patent No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, malate dehydrogenase, urease, peroxidase such as horseradish peroxidase (HRPO), alkaline phosphatase, -galactosidase, glucoamylase, lysozyme, saccharide oxidases (*e.g.*, glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase), heterocyclic oxidases (such as uricase and xanthine oxidase), lactoperoxidase, microperoxidase, and the like. Techniques for conjugating enzymes to antibodies are described in O'Sullivan *et al.*, Methods for the Preparation of Enzyme-Antibody Conjugates for use in Enzyme Immunoassay, in *Methods in Enzym.* (ed J. Langone & H. Van Vunakis), Academic press, New York, 73:147-166 (1981).

Examples of enzyme-substrate combinations include, for example:

(i) Horseradish peroxidase (HRPO) with hydrogen peroxidase as a substrate, wherein the hydrogen peroxidase oxidizes a dye precursor (*e.g.*, orthophenylene diamine (OPD) or 3,3',5,5'-tetramethyl benzidine hydrochloride (TMB));

(ii) alkaline phosphatase (AP) with para-Nitrophenyl phosphate as chromogenic substrate; and

(iii) -D-galactosidase (-D-Gal) with a chromogenic substrate (*e.g.*, p-nitrophenyl- -D-galactosidase) or fluorogenic substrate 4-methylumbelliferyl- -D-galactosidase.

Numerous other enzyme-substrate combinations are available to those skilled in the art. For a general review of these, see U.S. Patent Nos. 4,275,149 and 4,318,980.

Sometimes, the label is indirectly conjugated with the antibody. The skilled artisan will be aware of various techniques for achieving this. For example, the antibody can be conjugated with biotin and any of the three broad categories of labels mentioned above can be conjugated with avidin, or *vice versa*. Biotin binds selectively to avidin and thus, the label can be conjugated with the antibody in this indirect manner. Alternatively, to achieve indirect conjugation of the label with the antibody, the antibody is conjugated with a small hapten (*e.g.*, digoxin) and one of the different types of labels mentioned above is conjugated with an anti-hapten antibody (*e.g.*, anti-digoxin antibody). Thus, indirect conjugation of the label with the antibody can be achieved.

In another embodiment of the invention, the anti-trkC antibody need not be labeled, and the presence thereof can be detected using a labeled antibody which binds to the anti-trkC antibody.

The antibodies of the present invention may be employed in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays. Zola, *Monoclonal Antibodies: A Manual of Techniques*, pp.147-158 (CRC Press, Inc. 1987).

Competitive binding assays rely on the ability of a labeled standard to compete with the test sample analyte for binding with a limited amount of antibody. The amount of trkC protein in the test sample is inversely proportional to the amount of standard that becomes bound to the antibodies. To facilitate determining the amount of standard that becomes bound, the antibodies generally are insolubilized before or after the competition, so that the standard and analyte that are bound to the antibodies may conveniently be separated from the standard and analyte which remain unbound.

Sandwich assays involve the use of two antibodies, each capable of binding to a different immunogenic portion, or epitope, of the protein to be detected. In a sandwich assay, the test sample analyte is bound by a first antibody which is immobilized on a solid support, and thereafter a second antibody binds to the analyte, thus forming an insoluble three-part complex. See, *e.g.*, U. S. Patent No. 4,376,110. The second antibody may itself be labeled with a detectable moiety (direct sandwich assays) or may be measured using an anti-immunoglobulin antibody that is labeled with a detectable moiety (indirect sandwich assay). For example, one type of sandwich assay is an ELISA assay, in which case the detectable moiety is an enzyme.

The antibodies may also be used for *in vivo* diagnostic assays. Generally, the antibody is labeled with a radionuclide (such as ^{111}In, ^{99}Tc, ^{14}C, ^{131}I, ^{125}I, ^{3}H, ^{32}P or ^{35}S) so that the cells or tissue of interest can be localized using immunoscintiography.

The antibodies may also be used as staining reagents in pathology, following techniques well known in the art.

The anti-trkC agonist antibodies of the present invention are believed to possess numerous advantages over NT-3 as therapeutic agents, including improved efficacy, improved pharmacokinetic properties (pK) and bioavailability, and lack of hyperalgesia following administration.

4. Pharmaceutical formulations

Therapeutic formulations of the antibodies of the present invention are prepared for storage by mixing the antibody having the desired degree of purity with optional physiologically acceptable carriers, excipients or stabilizers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.*, Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

The formulations herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

The active ingredients may also be entrapped in microcapsule prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsule and poly-(methylmethacylate) microcapsule, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.*, films, or microcapsule. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the Lupron Depot™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as

ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

An effective amount of an antibody of the present invention to be employed therapeutically will depend, for example, upon the therapeutic objectives, the route of administration, and the condition of the patient. Accordingly, it will be necessary for the therapist to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. A typical daily dosage might range from about 1 g/kg to up to 100 mg/kg or more, depending on the factors mentioned above. Typically, the clinician will administer a molecule of the present invention until a dosage is reached that provides the required biological effect. The progress of this therapy is easily monitored by conventional assays.

Administration may be by any conventional route known in the art including, without limitation, intravenous, subcutaneous, topical, intramuscular, intratracheal, intracerebral, intranasal, intrapulmonary, and intraparyngal administration.

4. Gene therapy

The nucleic acid encoding the antibodies of the present invention may also be used in gene therapy of various (chronic) neurodegenerative disorders and acute nerve cell injuries, especially genetically acquired peripheral neuropathies. Two basic approaches to gene therapy have evolved: *ex vivo* gene therapy and *in vivo* gene therapy. In *ex vivo* gene therapy, cells are removed from a subject and cultured *in vitro*. A functional replacement gene is introduced into the cells *in vitro*, the modified cells are expanded in culture, and then reimplanted in the subject. In *in vivo* gene therapy, the target cells are not removed from the subject. Rather, the transferred gene is introduced into cells of the recipient *in situ*, that is, within the recipient.

Several *ex vivo* gene therapy studies in humans have been reported and are reviewed, for example, in Anderson, Science 256:808-813 (1992), and Miller, Nature 357:455-460 (1992).

The viability of *in vivo* gene therapy has been demonstrated in several animal models, as reviewed in Felgner *et al.*, Nature 349:351-352 (1991). Direct gene transfer has been reported, for example, into muscle tissue (Ferry *et al.*, Proc Natl. Acad. Sci. 88:8377-8781 (1991); Quantin *et al.*, Proc. Natl. Acad. Sci. USA 89:2581-2584 (1992)); the arterial wall (Nabel *et al.*, Science 244:1342-1344 (1989)); and the nervous system (Price *et al.*, Proc. Natl. Acad. Sci. 84:156-160 (1987)).

Accordingly, the present invention also provides delivery vehicles suitable for delivery of a polynucleotide encoding an agonist anti-trkC antibody into cells (whether *in vivo* or *ex vivo*). Generally, a polynucleotide encoding an

antibody (e.g. linear antibody or antibody chains) will be operably linked to a promoter and a heterologous polynucleotide. Delivery vehicles suitable for incorporation of a polynucleotide encoding an antibody of the present invention for introduction into a host cell include non-viral vehicles and viral vectors. Verma and Somia, Nature 389:239-242 (1997).

A wide variety of non-viral vehicles for delivery of a polynucleotide encoding an antibody of the present invention are known in the art and are encompassed in the present invention. A polynucleotide encoding an anti-trkC antibody can be delivered to a cell as naked DNA (U.S. Patent No. 5,692,622; WO 97/40163). Alternatively, a polynucleotide encoding an anti-trkC antibody herein can be delivered to a cell associated in a variety of ways with a variety of substances (forms of delivery) including, but not limited to cationic lipids; biocompatible polymers, including natural polymers and synthetic polymers; lipoproteins; polypeptides; polysaccharides; lipopolysaccharides; artificial viral envelopes; metal particles; and bacteria. A delivery vehicle can be a microparticle. Mixtures or conjugates of these various substances can also be used as delivery vehicles. A polynucleotide encoding an antibody herein can be associated non-covalently or covalently with these various forms of delivery. Liposomes can be targeted to a particular cell type, e.g., to a glomerular epithelial cell.

Viral vectors include, but are not limited to, DNA viral vectors such as those based on adenoviruses, herpes simplex virus, poxviruses such as vaccinia virus, and parvoviruses, including adeno-associated virus; and RNA viral vectors, including, but not limited to, the retroviral vectors. Retroviral vectors include murine leukemia virus, and lentiviruses such as human immunodeficiency virus. Naldini *et al.*, Science 272:263-267 (1996).

Non-viral delivery vehicles comprising a polynucleotide encoding an anti-trkC antibody can be introduced into host cells and/or target cells by any method known in the art, such as transfection by the calcium phosphate coprecipitation technique; electroporation; electropermeabilization; liposome-mediated transfection; ballistic transfection; biolistic processes including microparticle bombardment, jet injection, and needle and syringe injection; or by microinjection. Numerous methods of transfection are known to the skilled worker in the field.

Viral delivery vehicles can be introduced into cells by infection. Alternatively, viral vehicles can be incorporated into any of the non-viral delivery vehicles described above for delivery into cells. For example, viral vectors can be mixed with cationic lipids (Hodgson and Solaiman, Nature Biotechnol. 14:339-342 [1996]); or lamellar liposomes (Wilson *et al.* Proc. Natl. Acad. Sci. USA 74:3471 [1977]; and Faller *et al.* J. Virol. 49:269 [1984]).

In a preferred embodiment, nucleic acid encoding both the heavy and the light chains (including fragments) of an anti-trkC antibody of the present invention will be present in the same polycistronic expression vector, such as those disclosed in U.S. Patent Nos. 4,965,196 and 4,713,339. Polycistronic expression vectors contain sequences coding for a secondary protein and a desired protein, wherein both the desired and secondary sequences are governed by the same promoter. The coding sequences are separated by translational stop and start signal codons. The expression of the secondary sequence effects control over the expression of the sequence for the desired protein, and the secondary protein functions as a marker for selection of transfected cells.

In *in vivo* gene therapy, the vector may be administered to the recipient, for example, by intravenous (i.v.) injection. Suitable titers will depend on a variety of factors, such as the particular vector chosen, the host, strength of promoter used, and the severity of the disease being treated.

The invention will be further illustrated by the following non-limiting examples.

EXAMPLES

Example 1 Production and characterization of agonist anti-trkC monoclonal antibodies

Production and Isotyping of Antibodies

Wild type Balb/C mice and transgenic mice producing human IgG2 or IgG4 (Xenomice, described in Mendez *et al., Nature Genetics* 15: 146-156 [1997]) were hyperimmunized either intraperitoneally, via rear footpad, or subcutaneously with 20 µg of human trkC-IgG (Shelton *et al., J. Neurosci.* 15: 477-491 [1995]) in either Frieund's or Ribi adjuvant as described in Mendez et al. (*supra*). Spleen cells from the immune mice were fused with myeloma cells (X63.Ag8.653, ATCC Rockville, MD). A total of 33 fusions were performed using 253 Xenomice and 35 wild type Balb/C mice. Plates (21,734 wells total) were initially screened by direct ELISA using trkC-IgG. The ELISA screen identified 684 trkC positive hybridomas, all of which were then evaluated for agonist activity in trkC KIRA (Kinase activated Receptor Assay). The KIRA identified 14 Xenomouse derived and 22 wild type Balb/C mice derived hybridomas secreting anti-trkC agonist antibodies. These hybridomas were subcloned by limiting dilution, reassayed to confirm agonist activity, and were used to induce ascites by injecting into Pristane-primed Balb/C or nude mice (Hongo *et al., Hybridoma* 14: 253-260 [1995]). The monoclonal antibodies present in ascites were purified by Protein A affinity chromatography (Hongo *et al., supra*). Specific fusion efficiency (number of positives / number of wells screened) was 3% for both the Xenomouse and wild type Balb/C mouse fusions. The incidence of agonist monoclonal antibodies (agonists / number of trkC ELISA positives) was 3% and 8% for the Xenomouse and wild type Balb/C mouse fusions, respectively. Isotypes of the murine monoclonal antibodies were determined using either GIBCO BRL dipstick or Zymed mouse-typer isotyping kit, following supplier's instructions. The Xenomice were either IgG_2 or IgG_4 strain, producing corresponding isotypes of antibodies. Table 1 shows isotypes of various human and murine anti-trkC monoclonal antibodies. A total of 8 human IgG_2, 6 human IgG_4, 7 murine IgG_1, 10 murine IgG_{2a} and 5 murine IgG_{2b} monoclonal antibodies were identified. The monoclonal antibodies with the most potent agonist activity (depicted by asterisk in Table 2), as determined by KIRA assay, were selected for in-depth characterization.

Table 2

Human Mabs (14 Total)

IgG_2 Isotype (8 Mabs)	**IgG_4 Isotype (6 Mabs)**
2.5.1*	4.8

	6.1.2*	2337
	6.4.1*	2338
	2342	2339
	2343	2348
5	2344 *	2349*
	2345 *	
	2346	

10 **Murine Mabs (22 Total)**

IgG_1 (7)	IgG_{2a} (10)	IgG_{2b} (5)	IgG_3
2249	2248*	2252	
2250*	2272	2273	
2253*	2251	2277	
2254	2256	2279	
2256*	2274	2280	
2257	2275		
2260	2276		
	2278		
	2281		
	2282		

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Determination of Agonist Activity

a. *KIRA Assay*

Two bioassays were used to determine NT-3 agonist activity of anti-trkC monoclonal antibodies. The Kinase activated receptor assay (KIRA), which has been discussed in greater detail hereinabove, measures tyrosine phosphorylation of trkC in transfected cells in response to stimulation with a ligand, such as NT-3, or agonist monoclonal antibodies (Sadick *et al., Exp. Cell Res.* 234: 354-361 (1997)). The monoclonal antibodies were diluted to 27 µg/ml in KIRA stimulation buffer (F12/DMEM 50:50 containing 2% bovine serum albumin (BSA; Intergen Co., Purchase, NY) and 25 mM Hepes, 0.2 µm filtered). The monoclonal antibodies were further diluted serially 1:3 (8 dilutions total; concentrations ranging from 0.01-160 nM) in stimulation medium. Chinese Hamster Ovary (CHO) cells stably transfected with trkC fused with a 26 amino acid polypeptide flag epitope derived from HSV glycoprotein D (gD) were seeded (5×10^4 cells/well) and grown in 96-well cell culture plates. The cells were then stimulated with either NT-3 (as a positive control) or various anti-trkC monoclonal antibodies, using serial dilutions of 0.1; 1.56; 3.13; 6.25; 12.5; 25; 50 and 100 ng/ml. All dilutions were assayed in duplicate for 6 hours. The assay was carried out essentially as described in Sadick *et al.* (*supra*). Briefly, cells were lysed using Triton X-100 and trkC present in lysate captured in ELISA using antibodies against the gD epitope and phosphorylated trkC detected and quantitated using anti-phosphotyrosine antibodies suitably conjugated with enzyme. A monoclonal antibody not directed against trkC (anti-IL8 IgG_2 Xenomous-derived human antibody or anti-gp120 IgG, murine monoclonal antibody) was used as a negative

control. As shown in Figure 1 (A and B), all the selected anti-trkC monoclonal antibodies could mimic the activity of NT-3 inasmuch as they could stimulate tyrosine phosphorylation of trkC receptor. The human anti-trkC monoclonal antibodies (Fig. 1A) showed more potent agonistic activity than the murine anti-trkC monoclonal antibodies (Fig. 1B). For example, the best human anti-trkC monoclonal antibody is 10-fold more potent than the best murine anti-trkC monoclonal antibody. Furthermore, the human monoclonals were nearly as efficient as NT-3 especially in the lower range of concentration.

b. *PC12 neurite outgrowth assay*

Another assay used to determine NT-3 mimetic activity of anti-trkC monoclonal antibodies was PC12 neurite outgrowth assay. This assay measures the outgrowth of neurite processes by rat pheocytochroma cells (PC12) in response to stimulation by appropriate ligands. These cells express endogenous trkA and are therefore responsive to NGF. However, they do not express endogenous trkC and are therefore transfected with trkC expression construct in order to elicit response to NT-3 and its agonists. PC12 cells were transfected (Urfer *et al., Biochem.* 36:4775-4781 (1997); Tsoulfas *et al., Neuron* 10:975-990 (1993)) with full-length human trkC and plated in 96-well cell culture plates (1000 cells/well). Three days following transfection, anti-trkC monoclonal antibodies were added in triplicate (concentration ranging from 0.0002 to 2.7 nM) and incubated for an additional 3 days at 37°C. The cells were then analyzed by phase contrast microscopy and cells with neurites exceeding 2 times the diameter of the cell were counted. The human as well as the murine anti-trkC monoclonal antibodies could stimulate neurite outgrowth in PC12 cells as shown in Fig. 1 C and D. The human anti-trkC monoclonal antibodies (Fig. 1C) exhibited far more potent activity than the murine anti-trkC monoclonal antibodies (Fig. 1D) thus corroborating the results obtained in the KIRA assay. Furthermore, consistent with the KIRA assay results, the human anti-trkC monoclonal antibodies showed roughly similar stimulation as obtained with NT-3. The results obtained with the two bioassays described above demonstrate the ability of anti-trkC monoclonal antibodies to mimic the activity of NT-3, the natural ligand of trkC receptor.

Agonist activity of the monoclonal antibodies was ranked according to maximum induction of tyrosine phosphorylation and calculated EC50 of the phosphorylation curves in the KIRA assay and PC12 neurite outgrowth assay. Table 3 summarizes characteristics of various anti-trkC agonist monoclonal antibodies.

Table 3

MAb ID	Isotype	Agonist Activity KIRA/PC12	Binds Rat trkC	Immunoblot NR/Red.		Affinity Kd (nM)
Human MAbs				NR	Red.	
2.5.1	G2	(+++/+++)	NO	++	++	12
6.1.2	G2	(+++/++++)	NO	+	+	12.5

6.4.1	G2	(+++/+++)	YES	+	+	12
2344	G2	(+++/+++)	NO	++	+	19
2345	G2	(++++/++++)	NO	++	++	12.1
2349	G4	(++++/++++)	NO	++	+	23
Murine MAbs						
2248	G2a	(+/+)	NO	+++	-	5.9
2250	G1	(++/++)	NO	++	++	8.7
2253	G1	(++/+)	NO	++	++	42
2256	G1	(+/+)	YES	++	+	62

Testing Specificity of anti-trkC antibodies

The specificity of anti-trkC monoclonal antibodies was tested using direct ELISA. The microtiter plates were coated overnight with immunoadhesin construct of the receptor trkA-IgG, trkB-IgG or trkC-IgG as capture antigens (described in Shelton *et al., J. Neurosci.* 15: 477-491 [1995]) using 100 µl of 1 µg/ml solution diluted in 50 mM carbonate buffer, pH 9.5. CD4-IgG (Capon *et al., Nature* 337: 525-531 [1989]) was used in place of capture antigen as a negative control. The coated plates were incubated for 1 hr at room temperature with various concentration of anti-trkC monoclonal antibodies (100 µl of 0.01 to 1 µg/ml) diluted in PBS containing 0.5% BSA and 0.05% Tween 20. After washing to remove excess unbound antibodies, appropriate HRP conjugate (human monoclonal antibodies: goat anti-human κ-HRP, 1:5000 diluted; murine monoclonal antibodies: goat anti-mouse IgG (Fc)-HRP, 1:5000 diluted) was added and incubated for 1 hr at room temperature. The plates were then washed, developed and read as previously described (Hongo *et al., Hybridoma* 14: 253-260 [1995]). Figure 2 shows a representative example using a human anti-trkC monoclonal antibody 6.1.2. The binding was highly specific to trkC, and no significant cross-reaction was observed with either trkA or trkB. Similarly, other human and mouse anti-trkC monoclonal antibodies showed specific recognition of trkC.

The binding of various anti-trkC agonist monoclonal antibodies to human trkC and rat trkC was compared using a direct ELISA essentially as described above except the capture antigen used for human trkC was trkC-gD instead of trkC-IgG. Results shown in Figure 3 indicate that among human anti-trkC monoclonal antibodies, only 6.4.1 significantly recognized rat trkC, rest were specific for human trkC. Similarly, among murine monoclonal antibodies, only 2256 recognized rat trkC to a significant extent while others showed specific recognition of human trkC only.

Affinity studies

Affinities of anti-trkC agonist monoclonal antibodies were determined using using *BIAcore*-2000™ surface plasmon resonance (SPR) system (BIAcore, Inc., Piscataway, N.J.). CM5 biosensor chips were activated with N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier's instructions. In the first series of binding experiments, the antigen, gD-tagged trkC, was diluted into 10 mM sodium acetate buffer (pH 4.8), and injected over the activated chip at a concentration of 0.09 mg/mL. Using variable

exposure times, four ranges of antigen density were achieved: 14,000-17,000 response units (RU), 7000-9000 RU, 2000-3000 RU, and 400-600 RU. The chip was blocked with ethanolamine.

In the first series of kinetic measurements, anti-trkC antibodies (IgG's) were diluted into running buffer (PBS containing 0.05% Tween-20 and 0.01% sodium azide) and 0.03 mL (667 nM) was injected over the biosensor chip at 25°C at a flow rate of 0.01 mL/min. Regeneration was achieved with a 30 sec pulse of 10 mM HCl, followed by a 1 min pulse of 100 mM Tris-HCl, pH 8.0 and two wash steps.

In a second series of experiments, the IgG's (0.1 mg/mL in 10 mM sodium acetate, pH 4.8) were immobilized as described above, except that antibody density was limited to 1000-2000 RU. Two-fold serial dilutions of gD-trkC in the range of 3.7 M to 29 nM were then injected over the biosensor chip for kinetics measurements as described above.

The dissociation phase of each kinetic curve were fit to a single exponential dissociation rate (k_{off}), and these rates were used in the calculation of the association rate (k_{on}) from the injection phase, using a simple 1:1 Langmuir binding model (Lofas & Johnsson, 1990).

Equilibrium dissociation constants, K_d's, from SPR measurements were calculated as the ratio k_{off}/k_{on}.

The affinities of anti-trkC antibodies for gD-trkC were measured in SPR kinetics experiments with either antigen or antibody immobilized. Apparent affinities determined from experiments using low densities of immobilized antigen (0.4 to 0.6 ng/mm2), were generally consistent with those determined in experiments using immobilized IgG (see Table 2). However, at higher densities of immobilized gD-trkC, the apparent binding affinity of each antibody became progressively tighter by factors of as much as 10 fold, probably because of an avidity effect of binding by the bivalent IgG (data not shown). In some cases, no binding could be detected when trkC was injected over immobilized IgG. This may have occurred because immobilization of the IgG led to steric blocking of the antigen-binding site. Under all conditions tested, the antibody 2248 had the highest apparent affinity (K_d= 5.6 to 8.5 nM) of all antibodies tested.

Table 4

Binding affinities determined by SPR. Results are shown for IgG's binding to immobilized gD-trkC (400-600 RU) and for gD-trkC binding to immobilized IgG's (1000-3000 RU). NDB = no detectable binding.

	K_d (nM)	
Antibody (IgG)	Immobilized gD-trkC	Immobilized IgG
2248	5.9	8.5
2250	8.7	28
2253	42	51
2256	62	300
2344	19	NDB
2345	12	NDB
2349	23	NDB
6.4.1	12	28
6.1.2	13	16
2.5.1	12	NDB

Competition ELISA

A competition ELISA was used to get preliminary information about various groups to which these antibodies belong depending on the epitope(s) on trkC they recognize. In this assay, trkC-gD (1 μg/ml) was used as a capture antigen to coat microtiter plate. A specific biotinylated anti-trkC monoclonal antibody (1 μg/ml) was added to the coated plate either alone or in presence of another anti-trkC monoclonal antibody that was unlabeled and used in excess (50 μg/ml) as compared to the labeled antibody. If biotinylated antibody and unlabeled antibody both recognize the same or overlapping epitope, they will compete for binding to the immobilized trkC, resulting in decreased binding of the labeled antibody. If they recognize different and non-overlapping epitopes, there will be no competition between them, and the binding of the labeled antibody to the immobilized trkC will not be affected. Unlabeled human IgG2 and mouse IgG were used as negative control. A representative data in Figure 4 shows that all anti-trkC monoclonal antibodies, except murine anti-trkC 2248 monoclonal antibody, compete with labeled human anti-trkC 6.1.2 monoclonal antibody for binding to the immobilized trkC, suggesting that murine 2248 antibody recognizes an epitope on trkC that is different from the epitope(s) recognized by all other anti-trkC antibodies.

It is interesting to note that when unlabeled murine monoclonal antibody 2248 is bound first to immobilized trkC, none of the other (biotinylated) antibodies can access their binding site, suggesting that even though the epitopes are

distinct, steric hinderance may play a role. Such pairwise comparison gives valuable information and helps in classifying antibodies directed against the same antigen into different groups based on epitope recognition. A summary of such comparison is shown in Figure 5. The results indicate that the antibodies can be divided into two distinct groups: Group 1 encompasses all monoclonal antibodies except 2248, whereas Group 2 is composed of 2248.

Epitope Mapping with Domain Swap Mutants

Further epitope mapping was performed utilizing chimeric trkC in which various domains were replaced with corresponding domains from trkA or trkB. This approach was made possible by the fact that anti-trkC antibodies do not significantly cross-react with trkA or trkB. The use of such domain-swap mutants has a distinct advantage over deletion mutants. The deletion of a domain might disrupt the secondary structure of protein whereas substitution of a domain with a corresponding domain, of similar size and substantially similar amino acid sequence, from a related protein in domain-swap mutants is likely to retain the secondary structure. The extracellular domain of trk receptors is composed of 5 domains as shown in Figure 6A. D1 and D3 are cysteine-rich domains, D2 is a leucine-rich domain, and D4 and D5 are immunoglobulin-like domains. Domain-swap mutants of trkC containing replacement of D1, D4 and D5 with the corresponding domains from trkB or trkA were made (Urfer *et al., EMBO J.* 14:2795-2805 (1995)). Wild type trkC and wild type trkA were used as positive and negative controls respectively. The domain-swap mutants of trkC are designated according to the source of the replaced domain. For example, s1B has D1 domain from trkB, s4B has D4 domain from trkB, s5B has D5 domain from trkB, and s5A has D5 domain from trkA. All of the mutants were expressed as immunoadhesin, i.e. fused to IgG, and purified.

The binding of each of the agonist anti-trkC monoclonal antibody to various domain-swap mutants was evaluated by ELISA. $F(ab')_2$ fragment from goat anti-human IgG was used for coating microtiter plates to capture serial dilutions (100 µg/ml to 2.4 ng/ml, 100 µl/well, one hour at room temperature) of immunoadhesins (trkC-IgG, trkA-IgG and domain-swap mutants of trkC as immunoadhesin). Either unlabeled human or biotinylated murine anti-trkC monoclonal antibodies were added (100 µl per well; 1 µg/ml, one hour at room temperature) to the plates containing immobilized immunoadhesins, washed to remove unbound excess reagents, and incubated with goat anti-human κ or streptavidin conjugated to HRP. As shown in Figure 6B, all human anti-trkC monoclonal antibodies were able to recognize trkC domain-swap mutants with replacement of domain D1 or D4. However, replacement of domain D5 with the corresponding domain derived from either trkB or trkA destroyed recognition by anti-trkC antibodies. The extent of binding was reduced to the same low level as that observed with a negative control, trkA. The results suggest that all the human anti-trkC monoclonal antibodies tested recognize an epitope located somewhere in domain D5.

Similar analysis was performed with murine anti-trkC monoclonal antibodies essentially the same way except the secondary antibody used was goat anti-mouse IgG Fc coupled to HRP. As with the human anti-trkC antibodies, the replacement of domain D5 abolished binding to all the murine anti-trkC monoclonal antibodies tested (Figure 6B). Additionally, the replacement of domain D4 also destroyed the binding of 2248 murine anti-trkC antibody. The human

as well as murine anti-trkC agonist monoclonal antibodies all seem to recognize an epitope in domain 5 with the exception of 2248 murine antibody, which seems to additionally recognize a determinant in domain 4. It appears that 2248 epitope may be a linear epitope overlapping the boundary of domain 4 and 5. Alternatively, 2248 antibody might recognize a secondary structure formed by discontiguous epitope with determinants derived from both domain 4 and domain 5. Interestingly, Urfer *et al.* J. Biol Chem. 273: 5829-5840 [1998]) have earlier established the prominent role of domain 5 in trkC receptor for mediating the interaction with NT-3. Surprisingly, the antibodies described herein also bind to an epitope of trkC which is largely overlapping with that recognized by NT-3. This is surprising because of the relative sizes and shapes of NT-3 and immunoglobulin molecules. The likely mode of action of these activators is to crosslink the extracellular domains of two trkC molecules in such a way to bring together their intracellular tyrosine kinase domains and cross phosphorylate and activate them.

In homodimeric NT-3, it has been established that the two areas of the molecule which interact with trkC are diametrically opposed on opposite sides of the molecule, 180 degrees apart from each other. The distance between these areas is on the order of 16 Å. On the other hand, the two trkC interacting sites in the immunoglobulin molecules described here are not diametrically opposed. In addition to displaying the trkC binding domains at a different angle than NT-3, immunoglobulins will have the trkC binding domains separated from each other by a much wider distance than they are in NT-3. This will vary with the exact angle of the two Fab domains, but is in the range of 50 Å to 150 Å. It would have been difficult to have foreseen that two such very different crosslinkers as NT-3 and the agonist Mabs act as agonists when bound to the same site on trkC.

Site-Directed Mutagenesis

Site-directed mutagenesis approach was used to determine the contribution of selected individual amino acid residues of domain 5 in the recognition by anti-trkC antibodies. Figure 7 shows the amino acid sequence of human trkC domains 4 and 5. All dotted residues were mutagenized to alanine except residues L284, L286 and E287 which were changed to E, H, and K respectively (Urfer *et al., J. Biol. Chem.* 273: 5829-5840 [1998]). A total of 26 single amino acid mutations were made and evaluated for their effect on binding to anti-trkC monoclonal antibodies. The values shown in Table 5 represent the ratio of binding to anti-trkC antibody of mutant *vs* wildtype trkC. In order to minimize variation and provide effective comparison, EC50 values were determined for each mutant for each antibody and divided by the EC50 value obtained with wildtype trkC.

Table 5

trkC Mutant	NT-3*	2.5.1	6.1.2	6.4.1	2344	2345	2349	2248	2250	2253	2256	1436
trkC	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
R275A	1.1											
E280A	0.7											
E283A	1.7											

L284E	1.1	NB	NB	NB	NB	NB	NB	0.7	0.6	0.7	1.1	0.8
R285A	1.5	1.1	1.2	0.8	0.9	1.0	0.9	0.8	2.6	0.9	NB	0.4
L286H	1.2	0.6	1.3?	NB	0.9	0.5	0.6	0.6	0.4	0.4	0.6	0.6
E287K	27.3	NB	NB	NB	NB	NB	NB	0.6	0.8	0.7	0.7	0.6
E291A	1.0											
R295A	11.6											
Q309A	1.0											
R312A	0.8											
K315A	0.9											
H318A	1.0	0.8	1.1	0.7	0.8	1.0	0.7	1.0	1.0	0.8	0.8	1
E320A	1.0											
E324A	1.2											
E329A	1.0											
N335A	37.8	NB	NB	0.3	NB	NB	1.5	0.6	0.5	0.5	0.5	0.6
K336A	0.9											
T338A	30.3											
H339A	1.7											
K360A	1.0											
Q368A	1.2											
K366A	0.9											
E367A	1.2											
D372A	1.2											
E373A	1.1											

The gray areas indicate that the designated mutants did not have an initial effect on monoclonal antibody binding, and were therefore not re-assayed. Mutations that completely obliterated monoclonal antibody binding are shown as NB ("no binding observed"). The analysis indicates the major contribution of amino acid residues L284, E287 and N335 of trkC in recognition by anti-trkC agonist monoclonal antibodies tested. A model of the complex of trkC domain 5 with NT3 shows the position of these residues in close contact with CDRs of antibody (Figure 8). This model is based on the crustal structure of the complex of trkA domain 5 with NGF. For further details see, e.g. Urfer *et al. J. Biol. Chem.* (1998), *supra*, or Ultsch *et al.*, J. Mol. Biol. 290:149-159 (1999).

Cloning and Sequencing of Antibody Variable Regions

In order to better understand the molecular basis of interaction between trkC and anti-trkC monoclonal antibodies, the heavy and light chain variable sequences of agonist antibodies were cloned and DNA sequence determined. Total RNA was isolated from hybridoma cells producing the human and murine anti-trkC antibodies using RNA isolation kit from Stratagene (La Jolla, CA). RNA was reverse transcribed into cDNA using SuperScript II system (Life Technologies, Inc., Gaithersburg, MD) and specific 3' primers based on framework 4 sequences derived from the respective heavy or light chain subgroup (Kabat and Wu, *J. Immunol.* 147: 1709-1719 (1991)). Subsequent PCR amplification was performed using AmpliTaq DNA polymerase (Perkin Elmer, Foster City, CA) in presence of 2.5 M DMSO with specific forward primers based on the N-terminal amino acid sequences of heavy and light chains and the same 3' primers used for cDNA synthesis. PCR products were subcloned into an $F(ab)'_2$ vector containing both human

heavy and light chain constant regions (Carter *et al., Bio/Technology* 10: 163-167 [1992]). Five clones each of the V_H and V_L domains were sequenced and a consensus sequence was obtained.

Figure 9 shows the deduced amino acid sequences of heavy chain of anti-trkC agonist monoclonal antibodies (2250, SEQ ID NO: 42; 2253, SEQ ID NO: 43; 2256, SEQ ID NO: 44; 6.1.2, SEQ ID NO: 45; 6.4.1, SEQ ID NO: 46; 2345, SEQ ID NO: 47; and 2349, SEQ ID NO: 48). The deduced amino acid sequences of light chain of anti-trkC agonist monoclonal antibodies are shown in Figure 10 (2250, SEQ ID NO: 49; 2253, SEQ ID NO: 50; 2256, SEQ ID NO: 51; 6.1.2, SEQ ID NO: 53; 6.4.1, SEQ ID NO: 53; 2345, SEQ ID NO: 54; and 2349, SEQ ID NO: 55). In both Figure 9 and Figure 10 the Complementarity Determining Regions (CDRs) are labeled as CDR1, CDR2 and CDR3, and the corresponding amino acid residues are shown in bold face. Figure 11 summarizes the sequences of CDRs of heavy chain as well as light chain of various anti-trkC monoclonal antibodies along with designation of respective heavy and light chain variable family to which they belong.

Based on the determined amino acid sequences of the CDRs of the heavy and light chains of the anti-trkC agonist monoclonal antibodies, it is possible to provide a general formula for several of these regions. For the murine antibodies, the heavy chain CDR1 may be represented by the formula XaaWXaaXaaWVK (SEQ ID NO:37), wherein Xaa at position 1 is F or Y, Xaa at position 3 is I or M and Xaa at position 4 is E or H. The murine heavy chain CDR2 may be represented by the formula EIXaaPXaaXaaXaaTNYNEKFKXaa (SEQ ID NO:38), wherein Xaa at position 3 is L or Y, Xaa at position 5 is G or S, Xaa at position 6 is S or N, Xaa at position 7 is D or G, Xaa at position 8 is N or R and Xaa at position 17 is G or S. The murine heavy chain CDR3 may be represented by the formula KNRNYYGNYVV (SEQ ID NO:12) or KYYYGNSYRSWYFDV (SEQ ID NO:13). For the human antibodies, the heavy chain CDR1 may be represented by the formula XaaXaaXaaYYWXaa (SEQ ID NO:39), wherein Xaa at position 1 is S or I, Xaa at position 2 is G or S and Xaa at position 3 is G, T or Y and Xaa at position 7 is S or N. The human heavy chain CDR2 may be represented by the formula XaaIXaaXaaSGSXaaTXaaNPSLKS (SEQ ID NO:40), wherein Xaa at position 1 is Y or R, Xaa at position 3 is Y or F, Xaa at position 4 is Y or T, Xaa at position 8 is S or R and Xaa at position 10 is N orY. The human heavy chain CDR3 may be represented by DRDYDSTGDYYSYYGMDV (SEQ ID NO:14), DGGYSNPFD (SEQ ID NO:15) or the formula ERIAAAGXaaDYYYNGLXaaV (SEQ ID NO:41) wherein Xaa at position 8 is A or T and Xaa at position 16 is D or A.

The deduced amino acid sequence of heavy and light chain variable regions was confirmed by determination of N-terminal peptide sequence of these antibodies. Electroblotting onto Millipore Immobilon-PSQ membranes was carried out for 1 hr at 250 mA constant current in a BioRad Trans-Blot transfer cell (Matsudaira, *J. Biol. Chem.* 262: 10035-10038 [1987]). The PVDF membrane was stained with 0.1% Coomassie Blue R-250 in 50% methanol, 0.5 min, and destained for 2-3 min. with 10% acetic acid in 50% methanol. The mebrane was thoroughly washed with water and allowed to dry before storage at 20°C. Automated protein sequencing was performed on model 494A Perkin-Elmer sequencer (Perkin-Elmer Corporation, Foster City, CA) equipped with on-line PTH analyzer. Protein electroblotted onto PVDF membrane were sequenced in 6 mm micro glass cartridge. Peaks were integrated with Justice Innovation software using Nelson Analytical 760 interfaces. Sequence interpretation was performed on a DEC Alpha (Henzel *et*

al., J. Chromatography 404: 41-52 (1987)). Table 6 summarizes the classification of human and murine anti-trkC agonist monoclonal antibodies based on their N-terminal sequences.

Table 6

Human anti-trkC agonist mAbs	Heavy chain	Light chain
6.1.2	Subgroup II	Kappa I
6.4.1	Subgroup II	Kappa I
2345	Subgroup II	Kappa III
2349	Subgroup II	Kappa III
2.5.1	Subgroup II	Kappa I
2344	Subgroup II	Kappa I
Murine anti-trkC agonist mAbs		
2248	Subgroup IIA	Kappa I
2250	Subgroup IIA	Kappa I
2253	Subgroup IIA	Kappa IV
2256	Subgroup IIA	Kappa II

Example 2 Effect of agonist anti-trkC monoclonal antibodies on neuropathies in experimental animal model

The principal use of NT-3 agonists is in the treatment and/or prevention of peripheral neuropathies. It is known that large fiber myelinated sensory neurons, which are involved in mediating proprioception and vibration sense, express trkC that acts as a high affinity receptor for NT-3. Neuropathies involving these large fibers are common in diabetes and are also induced in response to certain chemotherapeutic agents particularly cisplatin and pyridoxine. NT-3 has shown efficacy in animal models of experimental diabetic neuropathy and cisplatin induced neuropathy. However, the use of NT-3 is severely hampered by its poor bioavailability as shown in a rodent model. The use of anti-trkC monoclonal antibodies as agonist of NT-3 offers numerous advantages and obviates a number of potential problems associated with the use of NT-3.

The *in vivo* half-life of agonist anti-trk monoclonal antibodies was determined by injecting either intravenously or subcutaneously in experimental animals. Shown on Figure 12 are serum levels of monoclonal antibody 2256 at various times after intravenous (IV) injection of 1 mg/kg or subcutaneous (subD) injection of 5 mg/kg in rats. The serum levels were determined b using the KIRA assay to measure the amount of fully functional antibody 2256 by its ability to increase tyrosine autophosphorylation of trkC. These data indicate that monoclonal antibody 2256 in the rat has a half-life of 9 days and a bioavailability of 69% after subcutaneous administration. These values are consistent with those obtained with other antibodies, and are distinctly different from those obtained with NT3. Also shown in Figure 12 is date obtained after injection of NT-3 at the same doses and routes as shown for Mab 2256 (1

mg/kg, IV; 5 mg/kg subQ). These data indicate a serum half-life on the order of 4-6 minutes for NT-3, and a subcutaneous bioavailability of 7%. These data indicate that the antibodies are a significant improvement over NT-3 in terms of the very important properties of bioavailability and *in vivo* serum half-life.

It has been shown in two animal models of large fiber sensory neuropathy that NT-3 can protect or reverse the effects of chemical insult. Very high doses of NT3 have been shown to protect large fiber sensory neurons from the toxic effects of high doses of pyridoxine, and more moderate doses of NT3 have been shown to reverse the effects of cisplatinum administration. Since there might be many differences in the tissue distribution of NT-3 and the agonist Mabs described here, it is important to determine whether the *in vitro* activity of the Mabs translates into efficacy in animal models.

In order to create an animal model of cisplatinum induced neuropathy, adult rats were dosed with cisplatinum twice a week for sixteen weeks with 1 mg/kg intraperitoneally (IP). At this point, rats were split into four groups. All four groups continued receiving cisplatinum twice weekly. In addition to the continued cisplatinum, one group received NT-3 at a dose of 1mg/kg, three times per week, one group received Mab 2256 at a dose of 1 mg/kg once a week, one group received Mab 6.4.1 at a dose of 1 mg/kg once a week, and one group received saline three times a week. The NT-3 doses were given subcutaneously, while the Mabs and saline were administered IV. This treatment regime was continued for an additional four weeks, for a total of twenty weeks of cisplatinum administration.

The function of large fiber sensory neurons was assessed in these animals electrophysiologically, by use of H-wave recording (Gao *et al.*, Ann. Neurol. 38(1):30-7 [1995]). As can be seen from the date shown in Figure 13, the sensory conducten velocity was very low in the animals treated with cisplatinum with saline alone. NT-3 treatment three times a week caused an improvement of this lowered conduction velocity, as did treatment with either Mab 2256 or Mab 6.4.1 once a week. The magnitude of the improvement seen with the monoclonal antibodies used once a week was at least as great as that seen with three times a week treament with NT-3.

Pyridoxine is also known to induce a sensory neuropathy that primarily damages the large myelinated subpopulation of sensory neurons (Helgren *et al.*, J. Neurosci. 17(1):372-82 [1997]). High doses of NT3 have been shown to block the development of this neuropathy (Helgren *et al., supra*). Treatment of animals with two different doses of pyridoxine (either 400 mg/kg or 600 mg/kg daily, IP) for two weeks causes damage to the large neurons of the DRG. This damage can be detected by a decrease in the expression of several proteins known to be expressed either preferentially or exclusively by large neurons in the DRG. The expression level of these markers was assessed by measuring the level of the mRNA encoding them by use of the TAQMAN RT-PCR technique.

Taqman RT-PCR for trkC agonist effects:

A. Probes and Primers

NFL

F-CAGCAGAACAAGGTCCTGCAA 21MER (SEQ ID NO: 72)
R-AGCGGGAAGGCTCTGAGTG 19MER (SEQ ID NO: 73)
P-AGCTGTTGGTGCTGCGCCAGAA 22MER (SEQ ID NO: 74)

NSE
F-TCCATTGAAGACCCATTCGAC 21MER (SEQ ID NO: 75)
R-GCCGACATTGGCTGTGAAC 19MER (SEQ ID NO: 76)
P-AGGATGACTGGGCAGCTTGGTCCA 24MER (SEQ ID NO: 77)

TRKC
F-CAGCCCACTGCACCATATCA 20MER (SEQ ID NO: 78)
R-CTGTATCCGGCCCAGCAT 18MER (SEQ ID NO: 79)
P-CCATGGCATCACTACACCTTCATCGGT 27MER (SEQ ID NO: 80)

CALRET
F-TGGGAAAATTGAGATGGCAGA 21MER (SEQ ID NO: 81)
R-GCTGCCTGAAGCACAAAAGG 20MER (SEQ ID NO: 82)
P-CGCAGATCCTGCCAACCGAAGAGA 24MER (SEQ ID NO: 83)

PARVALB.
F-GACACCACTCTTCTGGAAAATGC 23MER (SEQ ID NO: 84)
R-TTGCCAAACCAACACCTACCA 21MER (SEQ ID NO: 85)
P-ATCCGACACCACCTGTAGGGAGGACC 26MER (SEQ ID NO: 86)

GAPDH
F-CAGTGGCAAAGTGGAGATTGT 21MER (SEQ ID NO: 87)
R-AATTTGCCGTGAGTGGAGTC 20MER (SEQ ID NO: 88)
P-CCATCAACGACCCCTTCATTGACCTC 26MER (SEQ ID NO: 89)

Probes and primers were designed using Primer Express, (ABI-Perkin-Elmer). Guidelines for primer probe selection are included in Williams and Tucker (1999) PCR applications, pp. 365-75 (Academic Press).

B. Total RNA Preparation and Quantification

L4 and L5 were dissected from phosphate buffered saline perfused rats. Left and right sides were isolated in separate tubes. For total RNA used in standard curves, all DRG were dissected from control rats. Total RNA was isolated using the Qiagen Rneasy mini columns. Tissue was homogenized as per manufacturers instructions. Total RNA was quantified utilizing the Ribogreen Quantitation Kit (Molecular Probes) and following the manufacturers instructions.

C. RT-PCR

Twenty five nanograms of total RNA was used per 50ul reaction, except in standard curve reactions where 500, 250, 25 or 2.5 nanograms per reaction was used. Each reaction contained 25 pmol of each oligonucleotide primer, 0.2 mM of each dNTP, 100 nM flourescently labelled oligonucleotide probe, 1X RT-PCR buffer (PE biosystems), 2.0mM MgCl2, 20 U RNAse inhibitor, 12.5 MuLV reverse transcriptase (RT, PE biosystems) and 2.5U Amplitaq Gold polymerase (PE biosystems). Reverse transcription was performed for 30 min at 48 degrees C followed by 95 degrees C for 10 min for Amplitaq Gold activation and RT inactivation, then PCR; 40 cycles of 95 degrees C for 15 sec and 60 degrees C for one and a half minutes.

D. Gene Expression Quantitation

Control RNA was used to generate standard curves for a housekeeping gene and the genes of interest with each taqman run. A standard curve was obtained by plotting the threshold cycle (Ct) value obtained from the Taqman run versus the log of the quantity of control total RNA added. The resultant linear equation was solved for the log RNA value. Plugging in the experimental Ct value produced the log of the experimental gene expression value. Ten raised to the power of this value gives the experimental gene expression in nanograms.

As can be seen from Figure 14, pyridoxine treatment for two weeks resulted in a dose dependent decrease in neurofilament light chain (NFL), neuron specific enolase (NSE), trkC, and calretinin expression. Both the dose dependency and magnitude of these decreases varies from marker to marker, indicating a differential sensitivity of these proteins as markers of the neuronal damage.

In Figure 15 the results of treating animals with two doses of Mab 2256 along with the low dose (400 mg/kg daily) of pyridoxine are shown. NFL and NSE show a significant decrease in expression at this level of pyridoxine treatment. Cotreatment of animals with 5 mg/kg of Mab 2256 (subQ weekly) completely blocked this decrease in expression. A Mab 2256 dose of 1 mg/kg had no appreciable effect on the expression of these proteins. Neither trkC nor calretinin expression is significantly affected by this low dose pyridoxine treatment, but treatemtn with 5 mg/kg Mab 2256 actually increases trkC expression over control level.

When animals are treated with the higher pyridoxine dose of 800mg/kg daily, the expression of NFL, NSE and calretinin falls to very low levels, while trkC expression falls to about 50% of control values (Figure 16). Cotreatment with Mab 2256 at either 1 mg/kg or 5mg/kg significantly but not completely blocks the decrease in expression seen in trkC and calretinin. There is a slight trend towards protection seen with NFL and NSE expression in animals treated with Mab 2256, but it did not attain statistical significance. Thus, using multiple biochemical markers of damage to large sensory neurons, Mab 2256 is seen to be capable of ameliorating the toxicity of pyridoxine treatment.

In order to examine the electrophysiolgical and behavioral effects of pyridoxine neuropathy, rats were treated with twice daily injections of 400mg/kg pyridoxine for 8 days. The function of their large diameter sensory afferents ware tested electrophysiologically by recording the M-wave (direct motor) and H-wave (reflex sensory) response in the muscles of the foot after stimulation of the sciatic nerve at the thigh and the calf (Gao *et al.*, Ann. Neurol. 38(1):30-7 [1995]). Treatment with pyridoxine for 8 days resulted in a large decrease in the amplitude of the sensory response compared to the motor response as seen in Figure 18. Cotreatment with Mab 2256 significantly blocked the pyridoxine-induced decrease in the sensory amplitude. This is similar to effects published using very high doses (20mg/kg daily) of NT3 (Helgren *et al., supra*).

Animals treated with this regime of pyridoxine were also behaviorally tested for their proprioceptive function. They were trained to walk across a horizontal ladder in order to escape a bright light and white noise stimulus into a dark box. The animals were videotaped from below, and the quality of the placement of their hindpaws on the rungs of the ladder was read by an observer blind to their treatment. Each paw placement was scored as a good placement

(paw lands on forward part of metatarsals, immediately behind toes, with toes wrapping the rung immediately), solid landing (paw hits other than immediately behind toes, but solidly on rung, toes often not wrapping), near footfault (paw barely hits rung, either on the extreme forward part of toes or rearward aspect of heel, but does support weight) or footfault (paw either misses rung entirely or poor enough placement that foot does not support weight and falls through ladder upon weight bearing). Normal rats very quickly learn to place their hindpaws correctly, which requires an excellent proprioceptive sense of where the hindpaw is in space. After treatment with pyridoxine (400mg/kg twice daily for 8 days), the performance on this task had declined, with an almost thirty percent decline in good placements and an increase in both footfaults and near footfaults (Figure 18). Cotreatment of the animals with Mab 2256 during this time, allowed the animals to maintain a much higher degree of performance, with a smaller decline in good placements and smaller increases in footfaults and near footfaults.

In summary, cotreatment with Mab 2256 ameliorates the toxic effects of pyridoxine as measured biochemically, electrophysiologically, and by performance on a behavioral task.

After establishing that the trkC Mabs were therapeutically at least as effective as NT-3, the observed adverse event of hyperalgesia was examined. This side effect of NT-3 administration has been seen in rodents (see Figure 19) and in humans (Chaudhary *et al.*, Muscle and Nerve 23:189-192 [2000]). Rats were trained and tested for thermal sensitivity of the hind paws using a Hargreaves device and then administered 1mg/kg of Mab 2256 IV, or 1mg/kg NT-3 subcutaneously in the scruff. At two, four, and six hours after administration, the rats were again tested for their thermal withdrawal times. As can be seen from Figure 19, NT-3 administration caused a significant heat hyperalgesia at four and six hours post dosing, while the trkC Mab 2256 was without any effect on thermal pain sensation. So, at doses known to be effective in reversing or preventing neuropathyy, NT-3 does cause an increase in sensitivity to pain, while the Mab 2256 does not.

Cisplatin, a widely used chemotherapeutic agent, induces a sensory neuropathy with selective loss of vibration sense and proprioception. Here we demonstrate that neurotrophin-3 (NT-3), a member of the nerve growth factor family of neurotrophic factors, restored to normal levels the reduced H-reflex-related sensory nerve conduction velocity induced by cisplatin in rats. NT-3 treatment corrected an abnormal cytoplasmic distribution of neurofilament protein in large sensory neurons in dorsal root ganglia and the reduction in the numbers of myelinated fibers in sural nerves caused by cisplatin. The NT-3-dependent reversal of cisplatin neurotoxicity thus suggests the possible use of NT-3 in the treatment of peripheral sensory neuropathy.

Chronic treatment of adult rats for 2-3 weeks with high doses of pyridoxine (Vitamin B6) produced a profound proprioceptive loss, similar to that found in humans overdosed with this vitamin or treated with the chemotherapeutic agent cisplatin. Pyridoxine toxicity was manifest as deficits in simple and precise locomotion and sensory nerve function and as degeneration of large-diameter/large-fiber spinal sensory neurons. As assessed quantitatively in a beam-walking task and by EMG recording of H waves evoked by peripheral nerve stimulation, coadministration of the neurotrophic factor neurotrophin-3 (NT-3; 5-20 mg/kg/day, s.c.) during chronic pyridoxine treatment largely attenuated the behavioral and electrophysiological sequelae associated with pyridoxine toxicity.

Furthermore, NT-3 administration prevented degeneration of sensory fibers in the dorsal column of the spinal cord. These data are consistent with the evidence that NT-3 is a target-derived neurotrophic factor for muscle sensory afferents and suggest that pharmacological doses of NT-3 may be beneficial in the treatment of large-fiber sensory neuropathies.

Deposit of Biological Material

The following hybridoma cell lines and plasmids have been deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209, USA (ATCC) on June 21, 2000:

Hybridoma/Plasmid Designation	ATCC No.
2.5.1	PTA-2151
6.1.2	PTA-2148
6.4.1	PTA-2150
2344	PTA-2144
2345	PTA-2146
2349	PTA-2153
2248	PTA-2147
2250	PTA-2149
2253	PTA-2145
2256	PTA-2152
DNA pXCA-2250HL	PTA-2136
DNA pXCA-2253HL	PTA-2137
DNA pXCA-2256HL	PTA-2138
DNA pXCA-6.1.2H	PTA-2141
DNA pXCA-6.4.1H	PTA-2143
DNA pXCA-2345H	PTA-2142
DNA pXCA-2349H	PTA-2133
DNA vegf4chim-6.1.2L	PTA-2134
DNA vegf4chim-6.4.1L	PTA-2135
DNA vegf4chim-2345L	PTA-2139
DNA vegf4chim-2349L	PTA-2140

This deposit was made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of viable cultures for 30 years from the date of the deposit. The organisms will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Genentech, Inc. and

ATCC, which assures permanent and unrestricted availability of the progeny of the cultures to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 USC §122 and the Commissioner's rules pursuant thereto (including 37 CFR §1.12 with particular reference to 886 OG 638).

In respect of those designations in which a European patent is sought, a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample. (Rule 28(4) EPC)

The assignee of the present application has agreed that if the cultures on deposit should die or be lost or destroyed when cultivated under suitable conditions, they will be promptly replaced on notification with a viable specimen of the same culture. Availability of the deposited strain is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by the constructs deposited, since the deposited embodiments are intended to illustrate only certain aspects of the invention and any constructs that are functionally equivalent are within the scope of this invention. The deposit of material herein does not constitute an admission that the written description herein contained is inadequate to enable the practice of any aspect of the invention, including the best mode thereof, nor is it to be construed as limiting the scope of the claims to the specific illustrations that they represent. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

It is understood that the application of the teachings of the present invention to a specific problem or situation will be within the capabilities of one having ordinary skill in the art in light of the teachings contained herein. Examples of the products of the present invention and representative processes for their isolation, use, and manufacture appear below, but should not be construed to limit the invention.

All references cited throughout the specification and the references cited therein are hereby expressly incorporated by reference.

WHAT IS CLAIMED IS:

1. An agonist anti-trkC monoclonal antibody which

(a) shows no significant cross-reactivity with trkA or trkB; and

(b) recognizes an epitope in domain 5 of trkC.

2. The antibody of claim 1 which further recognizes an epitope in domain 4 of trkC.

3. The antibody of claim 1 which binds both human and rat trkC.

4. The antibody of claim 1 which is a human antibody.

5. The antibody of claim 1 which is a murine antibody.

6. The antibody of claim 5 which is humanized.

7. The antibody of claim 1 effective in the treatment of cisplatin- or pyridoxine-induced neuropathy.

8. The antibody of claim 1 effective in the treatment of diabetic neuropathy.

9. The antibody of claim 1 which does not cause hyperalgesia when administered to a patient.

10. The antibody of claim 1 which has increased bioavailability as compared to NT-3.

11. The antibody of claim 1 which has a higher specific activity than NT-3.

12. An anti-trkC antibody heavy chain comprising the following CDR's: a CDR1 selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4 and 5; a CDR2 selected from the group consisting of SEQ ID NOs: 6, 7, 8, 9, 10 and 11; and a CDR3 selected from the group consisting of SEQ ID NOs: 12, 13, 14, 15, 16 and 17.

13. An anti-trkC antibody light chain comprising the following CDR's: a CDR1 selected from the group consisting of SEQ ID NOs: 18, 19, 20, 21, 22, 23 and 24; a CDR2 selected from the group consisting of SEQ ID NOs: 25, 26, 27, 28, 29 and 30; and a CDR3 selected from the group consisting of SEQ ID NOs: 31, 32, 33, 34, 35 and 36.

14. A murine anti-trkC antibody heavy chain comprising the following CDR's:

(a) a CDR1 of the formula XaaWXaaXaaWVK (SEQ ID NO: 37), wherein Xaa at position 1 is F or Y; Xaa at position 3 is I or M; and Xaa at position 4 is E or H;

(b) a CDR2 of the formula EIXaaPXaaXaaXaaXaaTNYNEKFKXaa (SEQ ID NO: 38), wherein Xaa at position 3 is L or Y; Xaa at position 5is G or S; xaa at position 6 is S or N; Xaa at position 7 is D or G; Xaa at position 8 is N or R and Xaa at position 17 is G or S; and

(c) a CDR3 of the formula KNRNYYGNYVV (SEQ ID NO: 12) or KYYYGNSYRSWYFDV (SEQ ID NO: 13).

15. A human anti-trkC antibody heavy chain comprising the following CDR's:

(a) a CDR1 of the formula XaaXaaXaaYYWXaa (SEQ ID NO: 39), wherein Xaa at position 1 is S or I; Xaa at position 2 is G or S; Xaa at position 3 is G, T or Y, and Xaa at position 7 is S or N;

(b) a CDR2 of the formula XaaIXaaXaaSGSXaaTXaaNPSLKS (SEQ ID NO: 40), wherein Xaa at position 1 is Y or R; Xaa at position 3 is Y or F; Xaa at position 4 is Y or T; Xaa at position 8 is S or R; and Xaa at position 10 is N or Y; and

(c) a CDR3 of the formula selected from the group consisting of DRDYDSTGDYYSYYGMDV (SEQ ID NO: 14); DGGYSNPFD (SEQ ID NO: 15); ERIAAAGXaaDYYYNGLXaaV (SEQ ID NO: 41), wherein Xaa at position 8 is A or T and Xaa at position 16 is D or A.

16. An anti-trkC agonist monoclonal antibody comprising a heavy chain comprising the CDR's of the murine anti-trkC antibody heavy chain of claim 14 associated with a light chain.

17. The antibody of claim 16 comprising human framework residues.

18. The antibody of claim 17 which shows no significant cross-reactivity with trkA or trkB.

19. The antibody of claim 17 having a homo-tetrameric structure composed of two disulfide-bonded antibody heavy chain-light chain pairs.

20. The antibody of claim 17 which is an antibody fragment selected from the group consisting of Fv, Fab, Fab' and $F(ab')_2$ fragments.

21. An anti-trkC agonist monoclonal antibody comprising a human anti-trkC antibody heavy chain of claim 15 associated with a human antibody light chain.

22. The antibody of claim 21 which shows no significant cross-reactivity with trkA or trkB.

23. The antibody of claim 22 having a homo-tetrameric structure composed of two disulfide-bonded antibody heavy chain-light chain pairs.

24. The antibody of claim 22 which is an antibody fragment selected from the group consisting of Fv, Fab, Fab' and $F(ab')_2$ fragments.

25. The antibody of claim 22 which is an IgG.

26. The antibody of claim 25 which is an IgG-2 or IgG-4.

27. A murine anti-trkC agonist antibody selected from the group consisting of antibodies 2248, 2250, 2253 and 2258.

28. A human anti-trkC agonist antibody selected from the group consisting of antibodies 6.1.2, 6.4.1, 2345, 2349, 2.5.1 and 2344.

29. The antibody of claim 28 selected from the group consisting of antibodies 6.1.2, 6.4.1, 2345 and 2349.

30. An isolated nucleic acid molecule encoding a murine anti-trkC agonist antibody of claim 27.

31. An isolated nucleic acid molecule encoding a human anti-trkC agonist antibody of claim 28.

32. A nucleic acid molecule deposited with ATCC on June 21, 2000 under an accession number selected from the group consisting of PTA-2136, PTA-2137 and PTA-2138.

33. A nucleic acid molecule deposited with ATCC on June 21, 2000 under an accession number selected from the group consisting of PTA-2133, PTA-2134, PTA-2135, PTA-2139, PTA-2140, PTA-2141, PTA-2142 and PTA-2143.

34. A vector comprising a nucleic acid molecule of any one of claims 30-33.

35. A host cell line transformed with a nucleic acid molecule of any one of claims 30-33.

36. A hybridoma cell line transformed with a nucleic acid molecule of any one of claims 30-33.

37. An antibody produced by a hybridoma cell line of claim 36.

38. An isolated nucleic acid molecule encoding a heavy chain of an anti-trkC agonist monoclonal antibody, said nucleic acid molecule selected from the group consisting of SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO. 68 and SEQ ID NO: 70.

39. An isolated nucleic acid molecule encoding a light chain of an anti-trkC agonist monoclonal antibody, said nucleic acid molecule selected from the group consisting of SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69 and SEQ ID NO: 71.

40. A vector comprising a nucleic acid molecule of claim 38 or claim 39.

41. A host cell transformed with a nucleic acid molecule of claim 38 or claim 39.

42. A host cell transformed with both a nucleic acid molecule of claim 38 and a nucleic acid molecule of claim 39.

43. A hybridoma cell line transformed with a nucleic acid molecule of claim 38 or claim 39.

44. A hybridoma cell line transformed with a nucleic acid molecule of claim 38 and a nucleic acid molecule of claim 39.

45. An antibody produced by a hybridoma cell line of claim 43.

46. An antibody produced by a hybridoma cell line of claim 44.

47. A polypeptide encoded by a nucleic acid molecule of claim 38 or claim 39.

48. A polypeptide comprising polypeptide chains encoded by a nucleic acid molecule of claim 38 and a nucleic acid molecule of claim 39.

49. A pharmaceutical composition comprising an effective amount of an agonist anti-trkC monoclonal antibody of any one of claims 1, 16, and 21, in admixture with a pharmaceutically acceptable carrier.

50. A method for treating a neuropathy or neurodegenerative disease, or repairing a damaged nerve cell comprising administering to a mammal an effective amount of an antibody of claim 1.

51. The method of claim 50 wherein said neuropathy is selected from the group consisting of a peripheral neuropathy, diabetic neuropathy, and a large-fiber sensory neuropathy.

52. The method of claim 50 wherein said neurodegenerative disease is amyotrophic lateral sclerosis (ALS).

53. The method of claim 50 wherein said nerve cell is a sensory neuron or motor neuron.

54. The method of claim 53 wherein said sensory neuron is from dorsal root ganglia.

55. The method of claim 53 wherein said motor neuron is from the spinal cord.

56. The method of claim 50 wherein said administration is intravenous or subcutaneous.

57. The method of claim 50 wherein said administration is topical.

58. A method for enhancing the proliferation, maintenance or regeneration of peripheral neurons comprising contacting said neurons with an effective amount of an antibody of claim 1, claim 16, or claim 21.

59. A method for the treatment of a disease or condition involving cell degeneration in a mammalian subject by introducing nucleic acid encoding an anti-trkC antibody of claim 1 into a cell of such subject.

60. The method of claim 59 wherein the disease or condition selected from the group consisting of a neuropathy, a neurodegenerative disease, and nerve cell damage.

61. The method of claim 59 wherein said cell is a nerve cell.

62. The method of claim 59 wherein said nucleic acid is introduced into said cell *ex vivo*.

63. The method of claim 59 wherein said nucleic acid is introduced into said cell *in vivo*.

64. A method of inducing angiogenesis comprising delivering an anti-trkC antibody of claim 1 in an amount effective to induce angiogenesis.

65. A method of making an agonist anti-trkC antibody comprising preparing an antibody specifically binding trkC at a site overlapping with the binding site of a native NT-3 ligand of said trkC.

66. The method of claim 65 wherein said antibody binds trkC at essentially the same site as a native NT-3 ligand of said trkC.

67. The method of claim 65 wherein said antibody binds to an epitope within domain 5 of trk C.

68. The method of claim 67 wherein said trkC is a native human trkC polypeptide.

69. The method of claim 68 wherein said epitope includes amino acid residues L284, E287, and N335 of said native human trkC.

Figure 1A

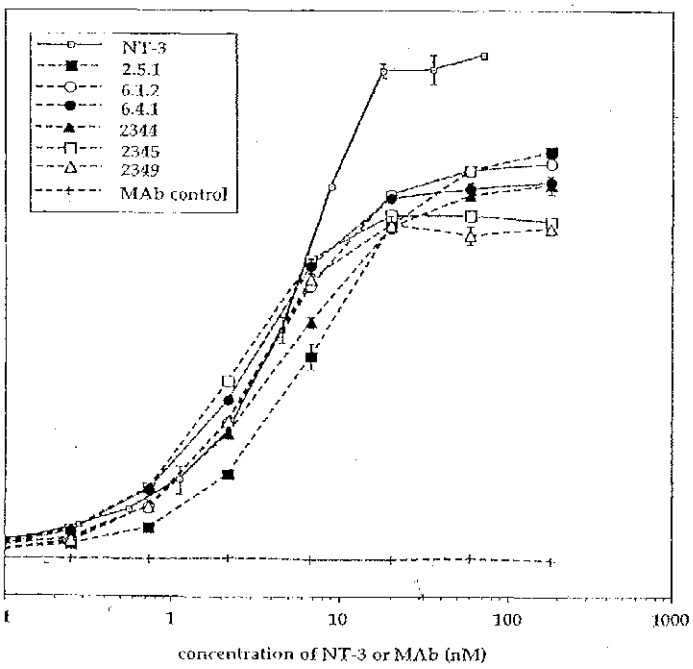

NT-3
2.5.1
6.4.2
6.4.1
2344
2345
2349
MAb control
1
10
100
1000
concentration of NT-3 or MAb (nM)

Figure 1B

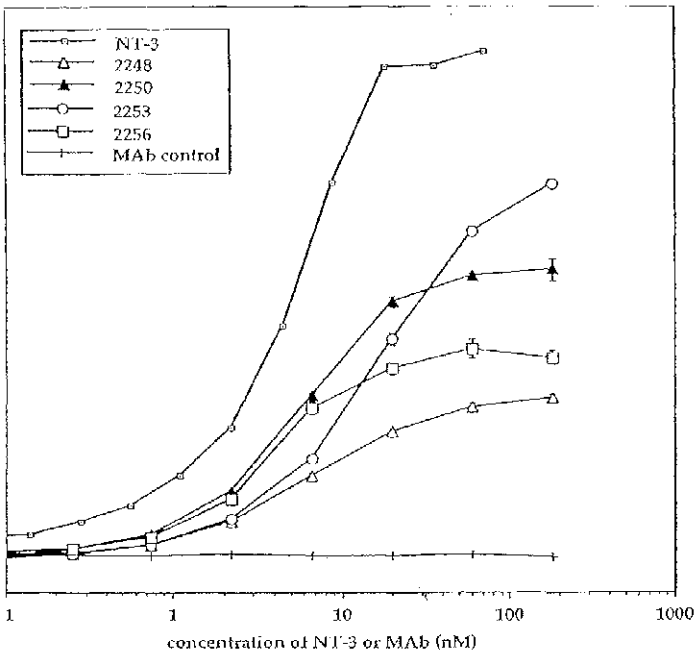

NT-3
2248
2250
2253
2256
MAb control
1
1
10
100
1000
concentration of NT-3 or MAb (nM)

Figure 1C

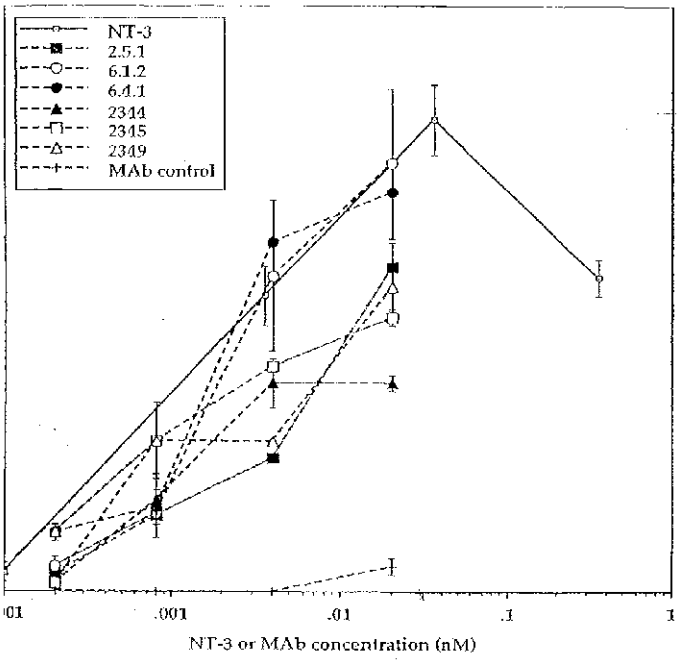

NT-3
2.5.1
6.1.2
6.4.1
2344
2345
2349
MAb control
.001
.01
.1
NT-3 or MAb concentration (nM)

Figure 1D

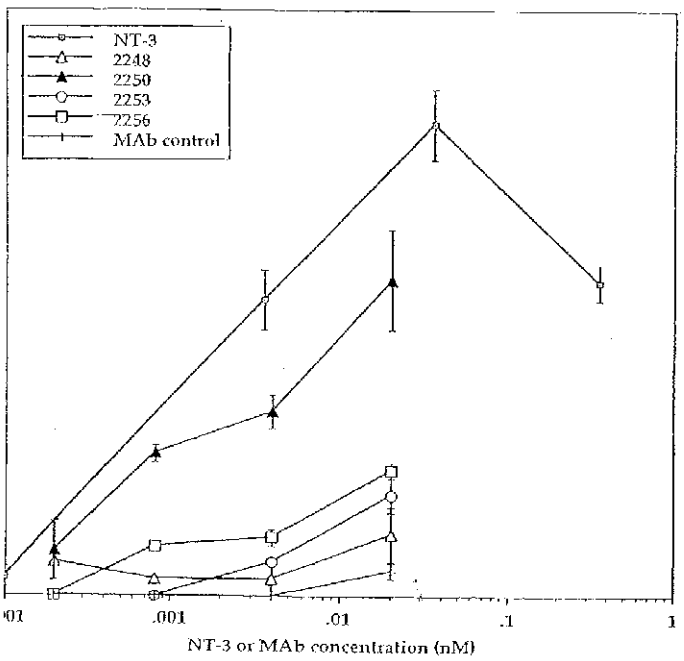
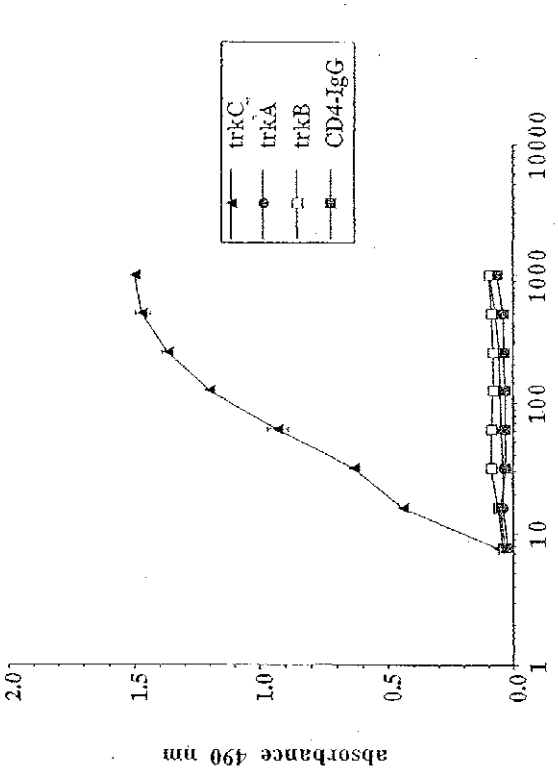

NT-3
2248
2250
2253
2256
MAb control
.001
.01
.1
1
NT-3 or MAb concentration (nM)

Fig. 2: The Agonist MAbs Bind SPECIFICALLY to trkC (6.1.2 shown as a representative MAb)



Assay Format: trk-IgG or CD4-IgG + MAb + appropriate HRP conjugate

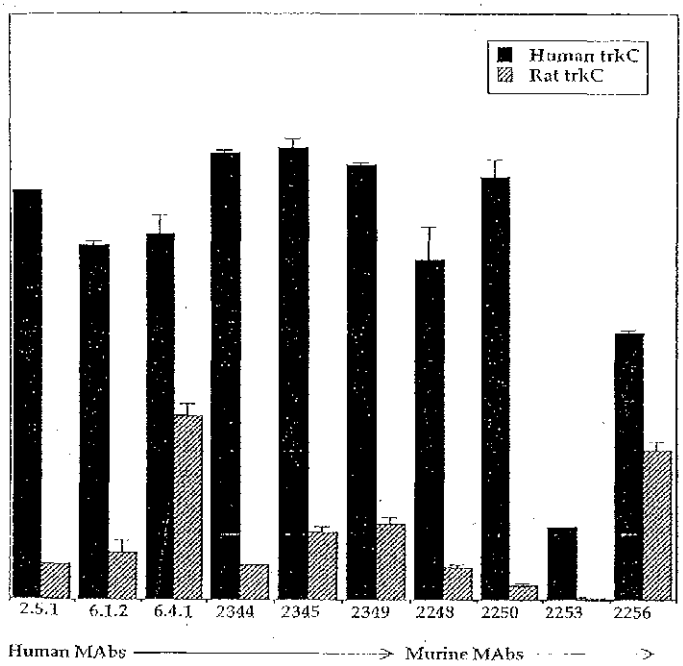

Human trkC
Rat trkC
2.5.1
6.1.2
6.4.1
2340
2345
2349
2248
2250
2253
2256
Human MAbs
Murine MAbs

Fig. 3

Fig. 4

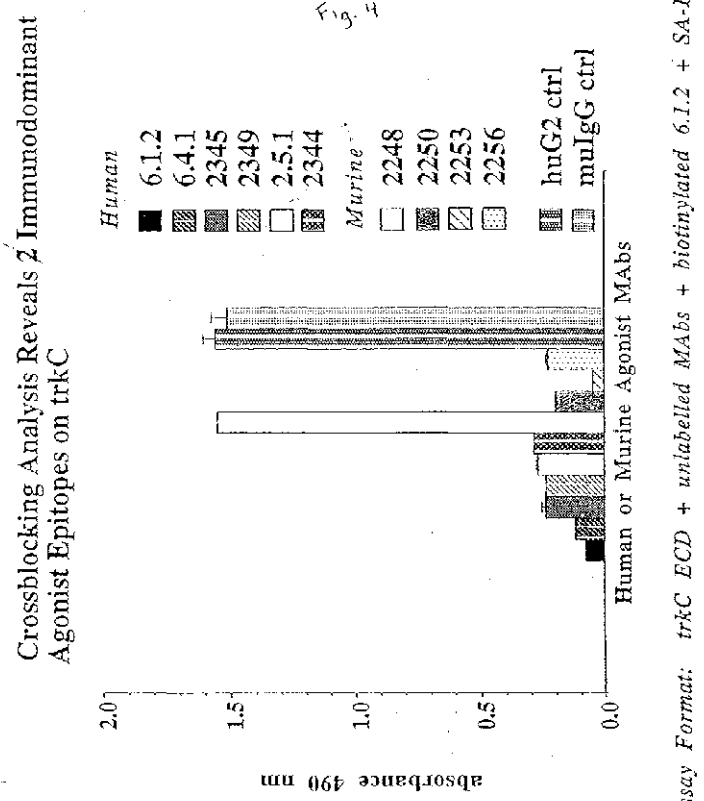

Crossblocking Analysis Reveals 2 Immunodominant Agonist Epitopes on trkC
Human
6.1.2
6.4.1
2345
2349
2.5.1
2344
Murine
2248
2250
2253
2256
huG2 ctrl
mulgG ctrl
absorbance 490 nm
2.0
1.5
1.0
0.5
0.0
Human or Murine Agonist MAbs
Assay Format: trkC ECD + unlabelled MAbs + biotinylated 6.1.2 + SA-HRP

Fig. 5

Epitope Mapping Summary
8/9/99

	unlabelled MAb									
	2344	2345	2349	2.5.1	5.1.2	6.4.1	2248	2250	2249.1	2266
Biotin-MAb										
2344	0.02	0	0	0.01	0	0	1.52	0	0	0
2345	0.28	0.04	0.03	0.17	0.11	3.09	1.48	0.04	0.07	0.35
2349	0.09	0.01	0	0.06	0.04	3.03	1.55	0.02	0.03	0.32
2.5.1	0.06	0.01	0.01	0.03	0.03	3.02	1.54	0.01	0.02	0.31
5.1.2	0.3	0.24	0.24	0.27	0.28	0.12	1.55	0.2	0.05	0.23
6.4.1	0.2	0.12	0.1	0.15	0.06	0.07	1.58	0.1	0.03	0.1
2248	1.52	1.63	1.54	1.6	0.74	1.72	0.07	1.4	0.04	1.48
2250	0.27	0.1	0.05	0.21	0.05	0.07	1.57	0.01	0.07	0.21
2249.1	1.35	1.4	1.45	1.4	0.46	0.8	1.55	1.24	0	1.34
2266	0.52	0.15	0.07	0.38	0.13	0.72	1.83	0.01	0.02	0.02

Group 1a	Group 1b	Group 2	Group 3
2344	2258	2248	2253*
2345	2252		
2349	2265		
2.5.1			
5.1.2			
6.4.1			
2250			
2266			

Domain mapping results are supportive of Group 1 & Group 2 division (2248 is the only MAb that binds Domains 4&5; others are D5 specific)
Point mutations suggest that the R318 is part of the epitope of 2253, 2250, and 2266

Assay format: gD-erbb + unlabeled mAb + biotinylated mAb + SA-HRP
(1 µg/ml) (50 µg/ml) (1 µg/ml) (1:1k)

Fig. 6A

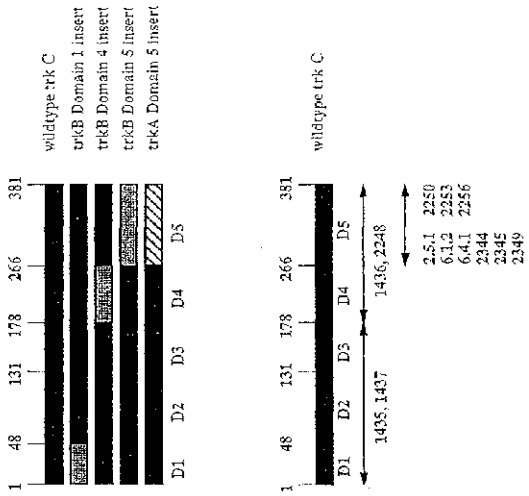
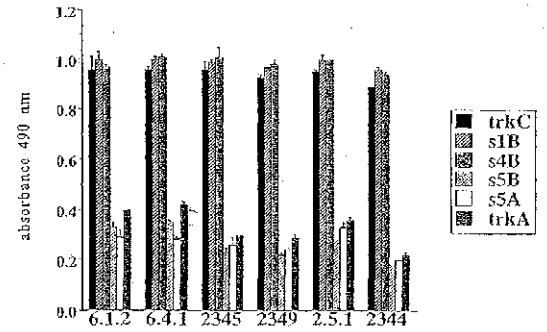


Fig. 6A: Ghost epitope mapping using trkA and trkB domain-swap mutants. The domain binding specificity of the MAbs was evaluated using whole domain replacement mutants. The following single domain substitutions into trkC were made: trkB domain 1, trkB domain 4, trkB domain 5 and trkA domain 5. Wildtype trkA was included as a negative control and previously epitope mapped non-agonist mutant MAbs (1435, 1436 and 1437) were included as MAb controls. Results of this mapping revealed Domain 5 binding specificity of all of the agonist MAbs, with the exception of MAb 2248, which binds an epitope formed by Domains 4 and 5.

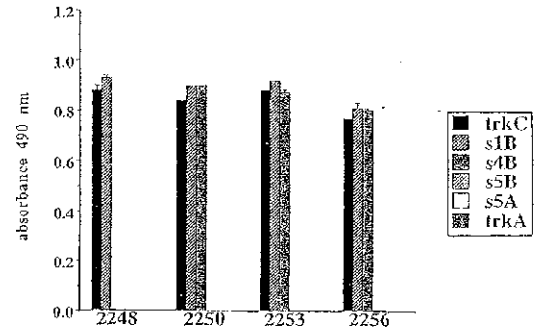
Fig. 5: Specific Binding of anti-trkC Agonist MAbs to Domain 5 of trkC



Assay Format:
goat F(ab')2 anti-huIgG Fc + trkC + MAb + goat anti-hu kappa-HRP

Fig. 6B

Fig. 5: Specific Binding of Murine anti-trkC Agonist MAbs to Either Domain 4 or 5 of trkC



Assay Format:
goat F(ab')2 anti-huIgG Fc + trkC-IgG + MAb + goat anti-mouse IgG Fc-H

Fig. 6C

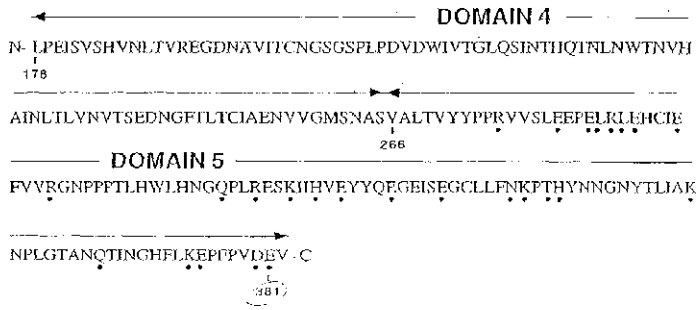


Figure 4: Sequence of hutrkC domains 4 and 5. All dotted residues were mutagenized to alanine except residues L284, L286, E287 which were changed to E, H, and K, respectively (/).

Fig. 7



Fig. 8

Fig. 9

Heavy chain sequences of anti-trkC agonist MAbs

CDR1 CDR2

```
2250 : N-QVQLQQSGAELMQPGASVKISCKSTGYTFSNFWIEWVKQRPG--HGLEWIGEILPGSDNTNYNEKFKG
2253 : N-QVQLQQSGAELMQPGASVKISCKSTGYTFSNFWIEWVKQRPG--HGLEWIGEILPGSDNTNYNEKFKG
2256 : N-QVQLQQPGAELVKPGASVKLSCKASGYTFTSYWMEWVKQRPG--QGLEWIGEIYPSNGRTNYNEKFKS

6.1.2: N-QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGGYYWSWIRQHPGKGLEWIGYIYYSG-STNYNPSLKS
6.4.1: N-QVQLQESGPGLVRPSETLSLTCTVSGG--SISTYYWNWIRQPAGKGLEWIGRIYTSG-STNYNPSLKS
2345 : N-QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGGYYWSWIRQHPEKGLEWIGYIFYSG-RTYYNPSLKS
2349 : N-QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGYYYWSWIRQHPQKGLEWIGYIYYSG-STYYNPSLKS
```

CDR3

```
2250 : KATFTADTSSNTAYMQLSSLTSEDSAVYYCARKN----RN----YYGNYVVAGQGTLVTVSA-C
2253 : KATFTADTSSNTAYMQLSSLTSEDSAVYYCARKN----RN----YYGNYVVAGAGTTLTVSS-C
2256 : KATLTVDKSSSTAYMQLSSLTSEDSAVYYCARKY----YYGNSYRSWYFDVWGAGTTLTVSS-C

6.1.2: RVTISVDTSKNQFSLKLSSVTAADTAVYYCTRDRDYDSTGDYYSYYGMDVWGQGTTVTVSS-C
6.4.1: RVTMSVDTSKNQFSLKLSSVTAADTAVYYCARDGYSNPFD---------WGQGTLVTVSS-C
2345 : RVTISVDTSKNQFSLKLNSVTAADTAVYYCA2ER-IAAAGADYYYNGLDVWGQGTTVTVSS-C
2349 : RLTISVDTSKNQFSLKLSSVTAADTAVYYCA2ER-IAAAGTDYYYNGLAVWGQGTTVTVSS-C
```

Fig. 10

Light chain sequences of anti-trkC agonist MAbs

CDR1 CDR2

2250 : N-DIVMTQSPASLAVSLGQRATISYRASKSVSTSG--YSYMHWNQQKPGQPPRLLIYLVSNLESGVPARF
2253 : N-QIVLTQSPAIMSASPGEKVTITCSASSSVSY------MYWYQQKPGTSPKLWIYSTSNLASGVPARF
2256 : N-DIVLTQSPASLAVSLGQRATISCRASESVDNYG--ISFMNWFQQKPGQPPKLLIYAASNQGSGVPARF

6.1.2 : N-DIQMTQSPSSLSASVGDRVTITCRASQGIRN------DLGWYQQKPGKAPKRLIYAASSLQSGVPSRF
6.4.1 : N-DIQMTQSPDSLAVSLGERATINCKSSQSVSYSSNNKNYLAWYQQKPGQPPKLLIYWASTRESGVPDRF
2345 : N-EIVLTQSPGTLSLSPGERATLSCRASQSVSS------NYLTWYQQKPGQAPRLLIYGASSRATGIPDRF
2349 : N-GIVLTQSPGTLSLSPGERATFSCRASQSGSS------TYLAWYQQKPGQAPRLLIYGASSRATGIPDRF

CDR3

2250 : SGSGSGTDFTLNIHPVEEEDAATYYCQHIRE-LTRSARGQSWKKR---C
2253 : SGSGSGTSYSLTISRMEAEDAATYYCQQRSS-YPLTFGAGTKLELKR-C
2256 : SGSGSGTDFSLNIHPMEEDDTAMYFCQQSKE-VPRTFGGGTKLEMKR-C

6.1.2 : SGSGSGTEFTLTISSLQPEDFATFYCLQHNS-LPLTFGGGTKVEIKR-C
6.4.1 : SGSGSGTDFTLTISSLQAEDVAVYYCQQHYN-TPLTFGGGTKVEIKR-C
2345 : SGSGSGTDFTLTISRLEPEDFAVYYCQQYGRSPITFGQGTRLEIKR-C
2349 : SGSGSGTDFTLTISRLEPEDFAVYYCQQYGRSPITFGQGTRLEIKR-C

Fig. 11

Antibody	Heavy chains				Light chains				
	Family	CDR1	CDR2	CDR3	Family	CDR1	CDR2	CDR3
Murine								
2250	VHIIA	FWISWVK	EILPGSGNTNYNEKFKG	KREV----YYGNYFDV	V KappaI	RASKSVSTSGYSYMH	LVSNLES	QHIRELTRS
2252	VHIIA	FWIEWVK	EILPGSGHTEYNEKFKG	KNEN----YYGNYFDY	V Kappa IV	SASSSVS-----YMY	STSNLAS	QQRSSYPLT
2256	VHIIA	YWMSWVK	EIYPGSGRTNYNEKFKS	KYYYGNSYRSWYFDV	V Kappa III	RASESVDNYGISFMN	AASNQGS	QQSKEVPWT
Human								
6.1.2	VHII	SGGYYWS	YIYYSGSTYYNPSLKS	DRDYDSTGDYYSYYGMDV	V KappaI	RASQGIRN------DLG	AASSLQS	LQHNS-LPLT
6.4.1	VHII	SSYYWSW	RIYTSGSTNYNPSLKS	DQSQWNRF2---------	V KappaI	KSSQSVSYSSSNNKNYLA	WASTRES	QQHYN-TPLT
2345	VHII	SGGYYWS	YIFYSGRTYYNPSLKS	ER--AAAGPDYYMGLDV	V KappaIII	RASQSVSS-----NYLT	GASSRAT	QQYGRSPPLT
2349	VHII	SGYYWS	YIYYSGSTYYNPSLKS	ER--AAAGTGYYMGLAV	V KappaIII	RASQSGSS-----YLA	GASSRAT	QQYGSSPPIT

Fig. 12

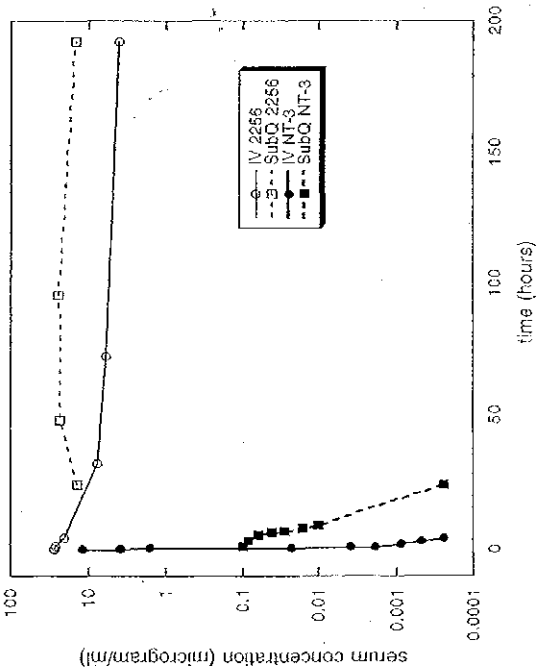


Figure 12. trkC agonist Mabs have improved half-life and bioavailability in vivo

Fig. 13

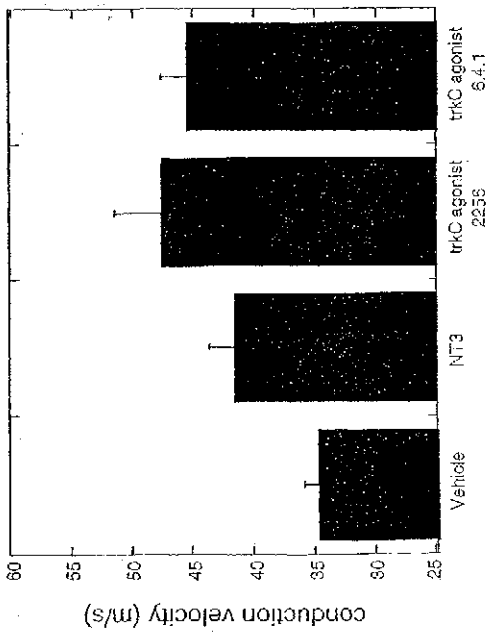


Figure 13. Treatment with trkC Agonists Reverses Cisplatinum-Induced Sensory Neuropathy

Fig. 14

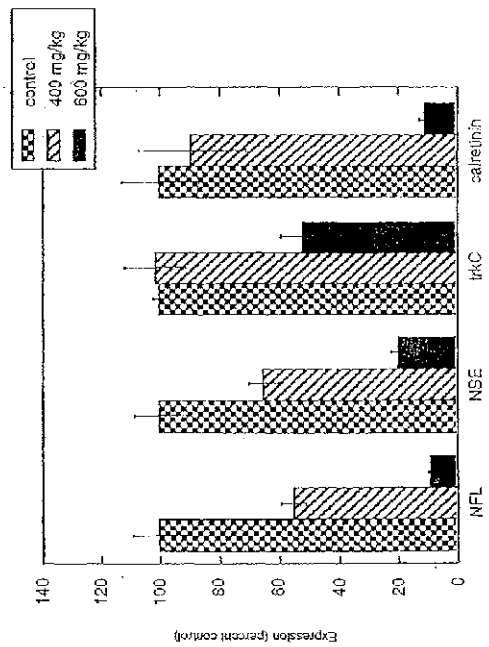
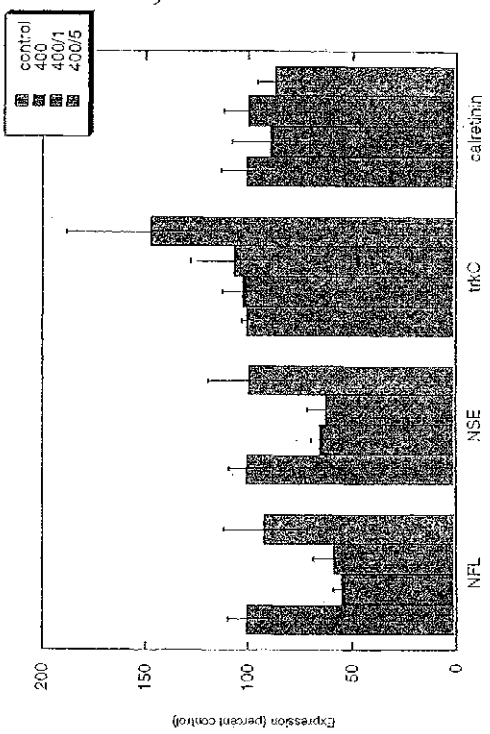


Figure 14. Pyridoxine neuropathy causes decreases in marker expression

Fig. 15

Agonist Mab ameliorates effects of low dose pyridoxine



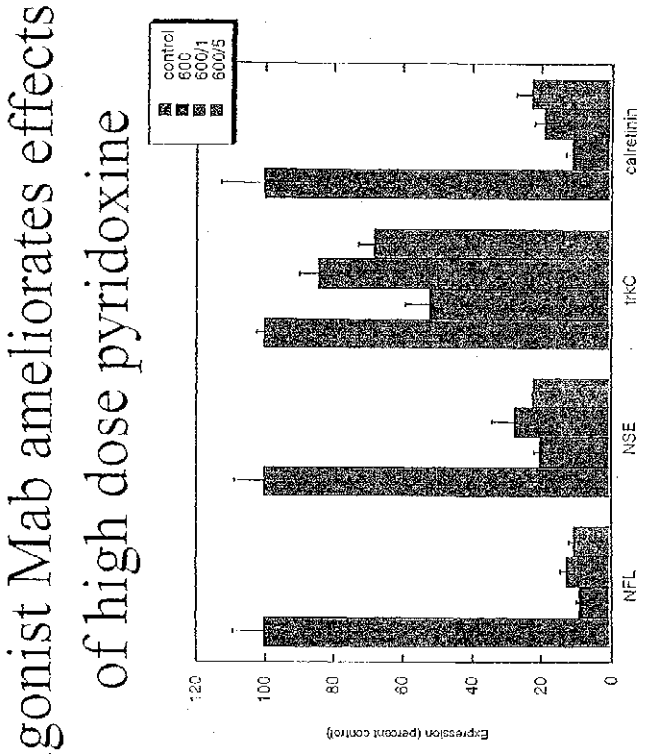

Agonist Mab ameliorates effects of high dose pyridoxine
control
600
600/1
600/5
Expression (percent control)
120
100
80
60
40
20
0
NFL
NSE
trkC
calretinin

Fig. 16

Fig. 17

Agonist antibody ameliorates pyridoxine neuropathy

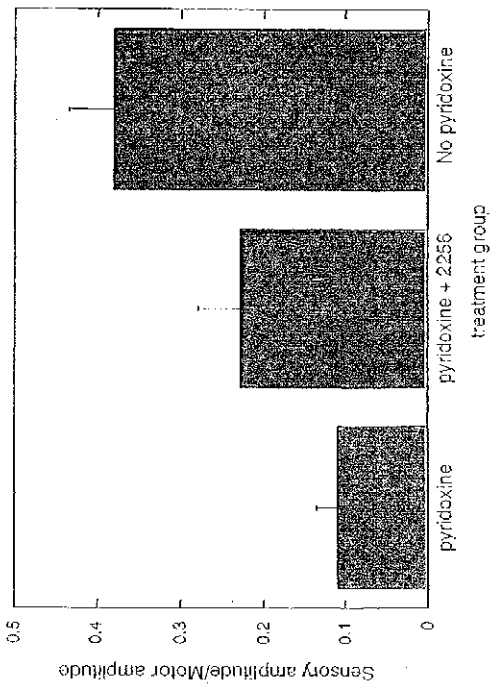


Fig. 18

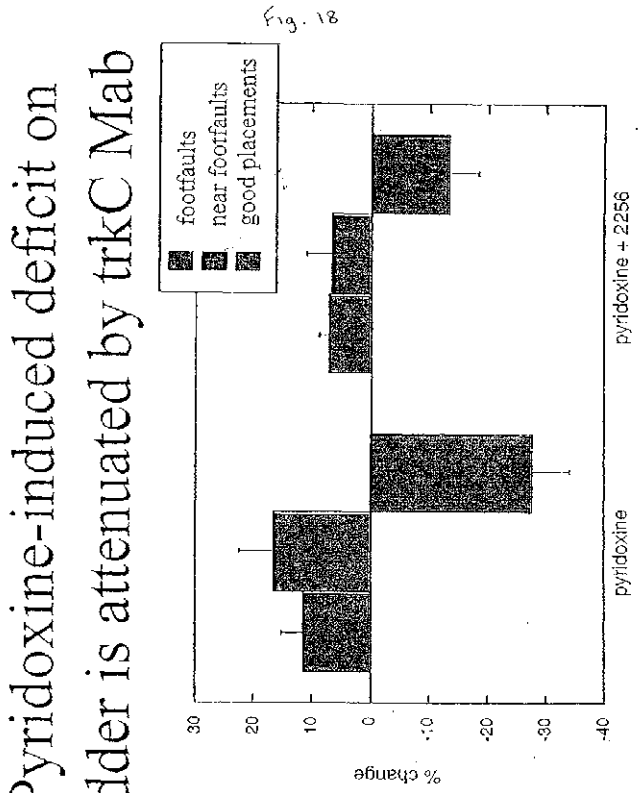

Pyridoxine-induced deficit on ladder is attenuated by trkC Mab
footfaults
near footfaults
good placements
% change
30
20
10
0
-10
-20
-30
-40
pyridoxine
pyridoxine + 2256

Fig. 19

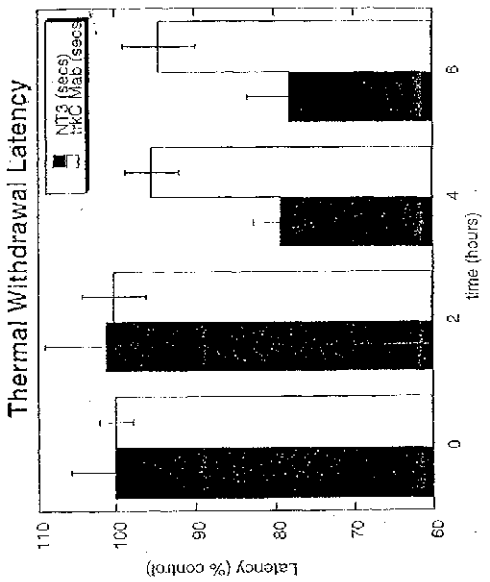


Figure 19. NT3, but not Mab, causes hyperalgesia at therapeutic doses

Fig. 20

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  101 IWLSSMRLTT LSWQLPDILS LAELQLSQNF FWCSGDIMAW QTAQSQSPSA LMSQWLKCSK PWNTSQTDLPK ISVSNVKSLCV RSSGNAVIPC

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  201 NNSGSPLPEV DWIVTGCQSS NTPKCPNLNT NYHAKNLTLV MYTSPDMGST LACKANNWVS NSNNSVALTV YYPPRVTSLE PPELRLENCI EFVVSGNPPP

                                                                                    /Start Domain 5

  301 TLAWQGAPGP LRSSKNIAVT YVGSGSISSG CLLFNKPWV RNGNFVTLAK NPLGTANQTI NGHFSAKPPP ESTDNFTLFD EVSPTPPITV THKPSEDTPG

                                                                                                    /End Domain 5

  401 VSIAVGLAAF AVLLIVGLAF WLWKVSRKSK PGMKSPVAVI SGEEGSASPL HHENSGITTF SSLDSQPGTV YSGMTRLPWL ENPQVPRQGA NCWKQSTTPL

  501 MLVRELIVLK KELGSGAFGK VFLAECYNLS PTKDKMLVAV KALKDPTLAA RKDFQREAEL LTNLQHEHIV KFYGVCVEGD PLIMVFEYMK HGDLNKFLRA

  601 HGPDAMILVD GQPRQAKGEL GLSQMLHIAS QIASGMVYLA SQHFVHRDLA TRNCLVGANL LVKIGDFGMS RDVYSTDYYR LGNRSVMPIR WMSPESVMYG

  701 KFSSKSDVWS FGVVLWEIFS LGYMPYPSKS NQEVLEFVTS GGRMDPPKNC PGPVYRIMTQ CWQHQPEDRP NFAIILERIE YCTQDPDVIN TALPIEYGPL

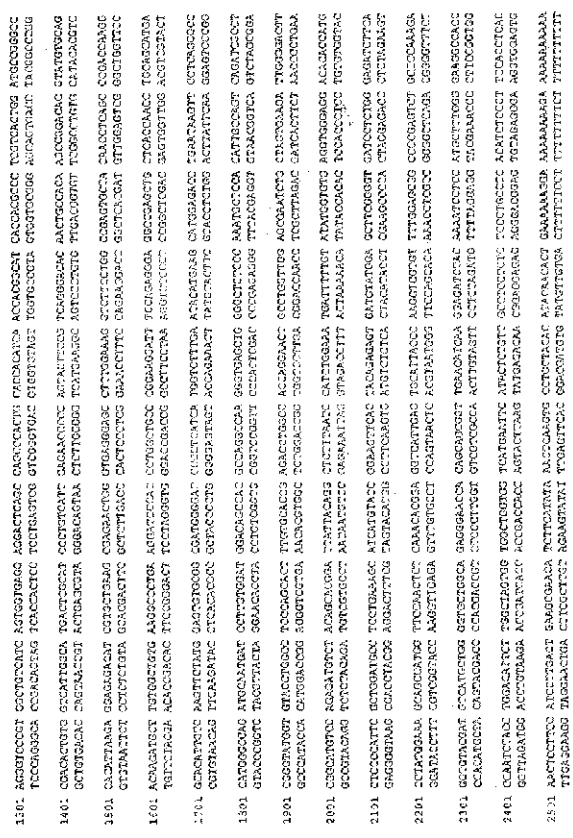
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Fig. 21

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v1.67 (Seq/Misc, Restrict, page 1

Fig. 21 (Pg. 2)



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2101 CTGCCCATTC GCTGGATTGC TCCTGAAGAC ATCATGTTAC CGGAGCTCAC AAGAAGAAGT GATCATATGA GCTTCGTCGT GAAGACTTCA
GACGGGTAAG CGACCTACGC AGGACTTTCG TAGTACAATG CCTTCGAGTG TCTTCTTCAC TTAGTATACT CGAAGCAGCA CTTCTGAAGT
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GATGCGATTG CAACCGTTAC GTTCGATGCT CTAGTTACCG ATGTTCGAGT GGCCGTGGTG GTTGAAGCTG TTCCAGAAGT AGCGGCCGAG GCCGTTGCTC
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ATGCAACTCAGCAGCCTGACATCTGAGGACTCTGCCGTCTATTACTGTGCAAGAAAGAAT
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B. MAb 2250 Light chain:

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GGGGTCCCTGCCAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACCCTCAACATCCAT
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TCGGCTCGGGGACAAAGTTGGAAAAAACGG

Fig 22

A. MAb 2253 Heavy chain:

CAGGTCCAGCTGCAGCAGTCTGGAGCTGAGCTGATGCAGCCTGGGGCCTCAGTGAAGATA
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B. MAb 2253 Light chain:

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Fig 23

A. MAb 2256 Heavy chain:

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CCTGGACAAGGCCTTGAGTGGATTGGAGAGATTTATCCTAGCAACGGTCGTACTAACTAC
AATGAGAAGTTCAAGAGCAAGGCCACACG
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TCTGCGGTCTATTACTGTGCAAGAAAATATTACTACGGTAATAGCTATCGTTCCTGGTAC
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B. MAb 2256 Light chain:

GACATTGTGCTGACCCAGTCTCCAGCTTCTTTGGCTGTGTCTCTAGGGCAGAGGGCCACC
ATCTCCTGCAGAGCCAGCGAAAGTGTTGATAATTATGGCATTAGTTTTATGAACTGGTTC
CAACAGAAACCAGGACAGCCACCCAAACTCCTCATCTATGCTGCATCCAACCAAGGATCC
GGGGTCCCTGCCAGGTTTAGTGGCAGTGGGTCTGGGACAGACTTCAGCCTCAACATCCAT
CCTATGGAGGAGGATGATACTGCAATGTATTTCTGTCAGCAAAGTAAGGAGGTTCCTCGG
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Fig 24

A. MAb 2345 Heavy chain:

CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCCTC
ACCTGCACTGTCTCTGGTGGCTCCATCAGCAGTGGTGGTTACTACTGGAGCTGGATCCGC
CAGCACCCAGAAAAGGGCCTGGAGTGGTTGGGTACATCTTTTACAGTGGGAGCACCTACT
ACAACCCGTCCCTCAAGAGTCGAGTTACATATCAGTAGACACGTCTAAGAACCAGTTCTC
CCTGAAGCTGAACTCTGTGACAGCCGCGGACACGGCCGTGTATTACTGTGCGAGAGAGCG
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B. MAb 2345 Light chain:

GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACC
CTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAGCAACTACTTAACCTGGTACCAGCAGAAA
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Fig 25

A. MAb 2349 Heavy chain:

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CCTGAAGCTGAGCTCTGTGACTGCCGCGGACACGGCCGTCTATTACTGTGCGAGAGAGCG
GATAGCAGCAGCTGGAACGGACTACTACTACAACGGTTTGGCCGTCTGGGGCCAAGGGAC
CACGGTCACCGTCTCCTCA

B. MAb 2349 Light chain:

GGAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACT
TTCTCCTGCAGGGCCAGTCAGAGTGGTAGCAGCACCTACTTAGCTGGTACCAGCAGAAAC
CTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAG
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CTGAAGATTTTGCAGTGTATTACTGTCAGCAGTATGGTAGGTCACCTCCGATCACCTTCG
GCCAAGGGACACGACTGGAGATTAAACGA

Fig 26

A. MAb 6.1.2 Heavy chain:

CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCCTC
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TACAACCCGTCCCTCAAGAGTCGAGTTACCATATCAGTGGACACGTCTAAGAACCAGTTC
TCCCTGAAGCTGAGCTCTGTGACTGCCGCGGACACGGCCGTGTATTACTGTACGAGAGAT
CGGGACTATGATAGTACCGGGGATTACTACTCCTACTACGGTATGGACGTCTGGGCCAA
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B. MAb 6.1.2 Light chain:

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Fig 27

A. MAb 6.4.1 Heavy chain:

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AAGCTGAGCTCTGTGACCGCCGCGGACACGGCCGTGTATTACTGTGCGAGAGATGGGGGC
TACAGTAACCCTTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA

B. MAb 6.4.1 Light chain:

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TGGTACCAGCAGAAACCTGGACAGCCTCCTAAGCTG
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Fig 28

SEQUENCE LISTING

<110> Genentech, Inc.
Devaux, Brigitte
Hongo, Jo-Anne S.
Presta, Leonard G.
Shelton, David L.

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Gln His Ile Arg Glu Leu Thr Arg Ser
1 5

<210> 32
<211> 9
<212> PRT
<213> Murine

<400> 32
Gln Gln Arg Ser Ser Tyr Pro Leu Thr
1 5

<210> 33
<211> 9
<212> PRT
<213> Murine

<400> 33
Gln Gln Ser Lys Glu Val Pro Arg Thr
1 5

<210> 34
<211> 9
<212> PRT
<213> Homo sapiens

<400> 34
Leu Gln His Asn Ser Leu Pro Leu Thr
1 5

<210> 35
<211> 9
<212> PRT
<213> Homo sapiens

<400> 35
Gln Gln His Tyr Asn Thr Pro Leu Thr
1 5

<210> 36
<211> 10
<212> PRT
<213> Homo sapiens

<400> 36
Gln Gln Tyr Gly Arg Ser Pro Pro Ile Thr
1 5 10

<210> 37
<211> 7
<212> PRT
<213> Murine

<220>
<221> UNSURE
<222> 1
<223> Xaa = F or Y

<221> UNSURE
<222> 3
<223> Xaa = I or M

<221> UNSURE
<222> 4
<223> Xaa = E or H

<400> 37
Xaa Trp Xaa Xaa Trp Val Lys
1 5

<210> 38
<211> 17
<212> PRT
<213> Murine

<220>
<221> UNSURE
<222> 3
<223> Xaa = L or Y

<221> UNSURE
<222> 5
<223> Xaa = G or S

<221> UNSURE
<222> 6
<223> Xaa = S or N

<221> UNSURE
<222> 7
<223> Xaa = D or S

<221> UNSURE
<222> 8
<223> Xaa = N or R

<221> UNSURE
<222> 17
<223> Xaa = G or S

<400> 38
Glu Ile Xaa Pro Xaa Xaa Xaa Xaa Thr Asn Tyr Asn Glu Lys Phe Lys
1 5 10 15
Xaa

<210> 39
<211> 7
<212> PRT
<213> Homo sapiens

<220>
<221> UNSURE
<222> 1
<223> Xaa = S or I

<221> UNSURE

<222> 2
<223> Xaa = G or S

<221> UNSURE
<222> 3
<223> Xaa = G, F or Y

<221> UNSURE
<222> 7
<223> Xaa = S or N

<400> 39
Xaa Xaa Xaa Tyr Tyr Trp Xaa
1 5

<210> 40
<211> 16
<212> PRT
<213> Homo sapiens

<220>
<221> UNSURE
<222> 1
<223> Xaa = Y or R

<221> UNSURE
<222> 3
<223> Xaa = Y or F

<221> UNSURE
<222> 4
<223> Xaa = Y or T

<221> UNSURE
<222> 8
<223> Xaa = S or R

<221> UNSURE
<222> 10
<223> Xaa = N or Y

<400> 40
Xaa Ile Xaa Xaa Ser Gly Ser Xaa Thr Xaa Asn Pro Ser Leu Lys Ser
1 5 10 15

<210> 41
<211> 17
<212> PRT
<213> Homo sapiens

<220>
<221> UNSURE
<222> 8
<223> Xaa = A or T

<221> UNSURE

<222> 16
<223> Xaa = D or A

<400> 41
Glu Arg Ile Ala Ala Ala Gly Xaa Asp Tyr Tyr Tyr Asn Gly Leu Xaa
1 5 10 15
Val

<210> 42
<211> 122
<212> PRT
<213> Murine

<400> 42
Asn Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Met Gln Pro Gly
1 5 10 15
Ala Ser Val Lys Ile Ser Cys Lys Ser Thr Gly Tyr Thr Phe Ser Asn
20 25 30
Phe Trp Ile Glu Trp Val Lys Gln Arg Pro Gly His Gly Leu Glu Trp
35 40 45
Ile Gly Glu Ile Leu Pro Gly Ser Asp Asn Thr Asn Tyr Asn Glu Lys
50 55 60
Phe Lys Gly Lys Ala Thr Phe Thr Ala Asp Thr Ser Ser Asn Thr Ala
65 70 75 80
Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr
85 90 95
Cys Ala Arg Lys Asn Arg Asn Tyr Tyr Gly Asn Tyr Val Val Trp Gly
100 105 110
Gln Gly Thr Leu Val Thr Val Ser Ala Cys
115 120

<210> 43
<211> 122
<212> PRT
<213> Murine

<400> 43
Asn Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Met Gln Pro Gly
1 5 10 15
Ala Ser Val Lys Ile Ser Cys Lys Ser Thr Gly Tyr Thr Phe Ser Asn
20 25 30
Phe Trp Ile Glu Trp Val Lys Gln Arg Pro Gly His Gly Leu Glu Trp
35 40 45
Ile Gly Glu Ile Leu Pro Gly Ser Asp Asn Thr Asn Tyr Asn Glu Lys
50 55 60
Phe Lys Gly Lys Ala Thr Phe Thr Ala Asp Thr Ser Ser Asn Thr Ala
65 70 75 80
Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr
85 90 95
Cys Ala Arg Lys Asn Arg Asn Tyr Tyr Gly Asn Tyr Val Val Trp Gly
100 105 110
Ala Gly Thr Thr Leu Thr Val Ser Ser Cys
115 120

<210> 44
<211> 126
<212> PRT
<213> Murine

<400> 44
Asn Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Lys Pro Gly
1 5 10 15
Ala Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser
20 25 30
Tyr Trp Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp
35 40 45
Ile Gly Glu Ile Tyr Pro Ser Asn Gly Arg Thr Asn Tyr Asn Glu Lys
50 55 60
Phe Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala
65 70 75 80
Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr
85 90 95
Cys Ala Arg Lys Tyr Tyr Tyr Gly Asn Ser Tyr Arg Ser Trp Tyr Phe
100 105 110
Asp Val Trp Gly Ala Gly Thr Thr Leu Thr Val Ser Ser Cys
115 120 125

<210> 45
<211> 130
<212> PRT
<213> Homo sapiens

<400> 45
Asn Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser
1 5 10 15
Gln Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Ser
20 25 30
Gly Gly Tyr Tyr Trp Ser Trp Ile Arg Gln His Pro Gly Lys Gly Leu
35 40 45
Glu Trp Ile Gly Tyr Ile Tyr Tyr Ser Gly Ser Thr Asn Tyr Asn Pro
50 55 60
Ser Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln
65 70 75 80
Phe Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Thr Arg Asp Arg Asp Tyr Asp Ser Thr Gly Asp Tyr Tyr Ser
100 105 110
Tyr Tyr Gly Met Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser
115 120 125
Ser Cys
130

<210> 46
<211> 119
<212> PRT
<213> Homo sapiens

<400> 46
Asn Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser
1 5 10 15

Glu Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Thr
20 25 30
Tyr Tyr Trp Asn Trp Ile Arg Gln Pro Ala Gly Lys Gly Leu Glu Trp
35 40 45
Ile Gly Arg Ile Tyr Thr Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu
50 55 60
Lys Ser Arg Val Thr Met Ser Val Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80
Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Asp Gly Gly Tyr Ser Asn Pro Phe Asp Trp Gly Gln Gly Thr
100 105 110
Leu Val Thr Val Ser Ser Cys
115

<210> 47
<211> 129
<212> PRT
<213> Homo sapiens

<400> 47
Asn Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser
1 5 10 15
Gln Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Ser
20 25 30
Gly Gly Tyr Tyr Trp Ser Trp Ile Arg Gln His Pro Glu Lys Gly Leu
35 40 45
Glu Trp Ile Gly Tyr Ile Phe Tyr Ser Gly Arg Thr Tyr Tyr Asn Pro
50 55 60
Ser Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln
65 70 75 80
Phe Ser Leu Lys Leu Asn Ser Val Thr Ala Ala Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Ala Arg Glu Arg Ile Ala Ala Ala Gly Ala Asp Tyr Tyr Tyr
100 105 110
Asn Gly Leu Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
115 120 125
Cys

<210> 48
<211> 129
<212> PRT
<213> Homo sapiens

<400> 48
Asn Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser
1 5 10 15
Gln Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Ser
20 25 30
Gly Tyr Tyr Trp Ser Trp Ile Arg Gln His Pro Gly Lys Gly Leu
35 40 45
Glu Trp Ile Gly Tyr Ile Tyr Tyr Ser Gly Ser Thr Tyr Tyr Asn Pro
50 55 60
Ser Leu Lys Ser Arg Leu Thr Ile Ser Val Asp Thr Ser Lys Asn Gln
65 70 75 80

Phe Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Ala Arg Glu Arg Ile Ala Ala Ala Gly Thr Asp Tyr Tyr Tyr
100 105 110
Asn Gly Leu Ala Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
115 120 125
Cys

<210> 49
<211> 112
<212> PRT
<213> Murine

<400> 49
Asn Asp Ile Val Met Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu
1 5 10 15
Gly Gln Arg Ala Thr Ile Ser Tyr Arg Ala Ser Lys Ser Val Ser Thr
20 25 30
Ser Gly Tyr Ser Tyr Met His Trp Asn Gln Gln Lys Pro Gly Gln Pro
35 40 45
Pro Arg Leu Leu Ile Tyr Leu Val Ser Asn Leu Glu Ser Gly Val Pro
50 55 60
Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile
65 70 75 80
His Pro Val Glu Glu Glu Asp Ala Ala Thr Tyr Tyr Cys Gln His Ile
85 90 95
Arg Glu Leu Thr Arg Ser Ala Arg Gly Gln Ser Trp Lys Lys Arg Cys
100 105 110

<210> 50
<211> 109
<212> PRT
<213> Murine

<400> 50
Asn Gln Ile Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro
1 5 10 15
Gly Glu Lys Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr
20 25 30
Met Tyr Trp Phe Gln Gln Lys Pro Gly Thr Ser Pro Lys Leu Trp Ile
35 40 45
Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg Met Glu Ala
65 70 75 80
Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Arg Ser Ser Tyr Pro Leu
85 90 95
Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg Cys
100 105

<210> 51
<211> 114
<212> PRT
<213> Murine

<400> 51
Asn Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu
1 5 10 15
Gly Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Asp Asn
20 25 30
Tyr Gly Ile Ser Phe Met Asn Trp Phe Gln Gln Lys Pro Gly Gln Pro
35 40 45
Pro Lys Leu Leu Ile Tyr Ala Ala Ser Asn Gln Gly Ser Gly Val Pro
50 55 60
Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu Asn Ile
65 70 75 80
His Pro Met Glu Glu Asp Asp Thr Ala Met Tyr Phe Cys Gln Gln Ser
85 90 95
Lys Glu Val Pro Arg Thr Phe Gly Gly Gly Thr Lys Leu Glu Met Lys
100 105 110
Arg Cys

<210> 52
<211> 110
<212> PRT
<213> Homo sapiens

<400> 52
Asn Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15
Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn
20 25 30
Asp Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu
35 40 45
Ile Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser
50 55 60
Gly Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln
65 70 75 80
Pro Glu Asp Phe Ala Thr Phe Tyr Cys Leu Gln His Asn Ser Leu Pro
85 90 95
Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Cys
100 105 110

<210> 53
<211> 116
<212> PRT
<213> Homo sapiens

<400> 53
Asn Asp Ile Gln Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu
1 5 10 15
Gly Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Ser Tyr
20 25 30
Ser Ser Asn Asn Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly
35 40 45
Gln Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly
50 55 60
Val Pro Asp Arg Ile Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu
65 70 75 80

Thr Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln
85 90 95
Gln His Tyr Asn Thr Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu
100 105 110
Ile Lys Arg Cys
115

<210> 54
<211> 112
<212> PRT
<213> Homo sapiens

<400> 54
Asn Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro
1 5 10 15
Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser
20 25 30
Asn Tyr Leu Thr Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu
35 40 45
Leu Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe
50 55 60
Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu
65 70 75 80
Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Arg Ser
85 90 95
Pro Pro Ile Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys Arg Cys
100 105 110

<210> 55
<211> 112
<212> PRT
<213> Homo sapiens

<400> 55
Asn Gly Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro
1 5 10 15
Gly Glu Arg Ala Thr Phe Ser Cys Arg Ala Ser Gln Ser Gly Ser Ser
20 25 30
Thr Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu
35 40 45
Leu Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe
50 55 60
Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu
65 70 75 80
Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Arg Ser
85 90 95
Pro Pro Ile Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys Arg Cys
100 105 110

<210> 56
<211> 808
<212> PRT
<213> Homo sapiens

<400> 56

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Cys Pro Ala Asn Cys Val Cys Ser Lys Thr Glu Ile Asn Cys Arg Arg
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Pro Asp Asp Gly Asn Leu Phe Pro Leu Leu Glu Gly Gln Asp Ser Gly
            20                  25                  30
Asn Ser Asn Gly Asn Ala Asn Ile Asn Ile Thr Asp Ile Ser Arg Asn
        35                  40                  45
Ile Thr Ser Ile His Ile Glu Asn Trp Arg Ser Leu His Thr Leu Asn
    50                  55                  60
Ala Val Asp Met Glu Leu Tyr Thr Gly Leu Gln Lys Leu Thr Ile Lys
65                  70                  75                  80
Asn Ser Gly Leu Arg Ser Ile Gln Pro Arg Ala Phe Ala Lys Asn Pro
                85                  90                  95
His Leu Arg Tyr Ile Asn Leu Ser Ser Asn Arg Leu Thr Thr Leu Ser
            100                 105                 110
Trp Gln Leu Phe Gln Thr Leu Ser Leu Arg Glu Leu Gln Leu Glu Gln
        115                 120                 125
Asn Phe Phe Asn Cys Ser Cys Asp Ile Arg Trp Met Gln Leu Trp Gln
    130                 135                 140
Glu Gln Gly Glu Ala Lys Leu Asn Ser Gln Asn Leu Tyr Cys Ile Asn
145                 150                 155                 160
Ala Asp Gly Ser Gln Leu Pro Leu Phe Arg Met Asn Ile Ser Gln Cys
                165                 170                 175
Asp Leu Pro Glu Ile Ser Val Ser His Val Asn Leu Thr Val Arg Glu
            180                 185                 190
Gly Asp Asn Ala Val Ile Thr Cys Asn Gly Ser Gly Ser Pro Leu Pro
        195                 200                 205
Asp Val Asp Trp Ile Val Thr Gly Leu Gln Ser Ile Asn Thr His Gln
    210                 215                 220
Thr Asn Leu Asn Trp Thr Asn Val His Ala Ile Asn Leu Thr Leu Val
225                 230                 235                 240
Asn Val Thr Ser Glu Asp Asn Gly Phe Thr Leu Thr Cys Ile Ala Glu
                245                 250                 255
Asn Val Val Gly Met Ser Asn Ala Ser Val Ala Leu Thr Val Tyr Tyr
            260                 265                 270
Pro Pro Arg Val Val Ser Leu Glu Glu Pro Glu Leu Arg Leu Glu His
        275                 280                 285
Cys Ile Glu Phe Val Val Arg Gly Asn Pro Pro Pro Thr Leu His Trp
    290                 295                 300
Leu His Asn Gly Gln Pro Leu Arg Glu Ser Lys Ile Ile His Val Glu
305                 310                 315                 320
Tyr Tyr Gln Glu Gly Glu Ile Ser Glu Gly Cys Leu Leu Phe Asn Lys
                325                 330                 335
Pro Thr His Tyr Asn Asn Gly Asn Tyr Thr Leu Ile Ala Lys Asn Pro
            340                 345                 350
Leu Gly Thr Ala Asn Gln Thr Ile Asn Gly His Phe Leu Lys Glu Pro
        355                 360                 365
Phe Pro Glu Ser Thr Asp Asn Phe Ile Leu Phe Asp Glu Val Ser Pro
    370                 375                 380
Thr Pro Pro Ile Thr Val Thr His Lys Pro Glu Glu Asp Thr Phe Gly
385                 390                 395                 400
Val Ser Ile Ala Val Gly Leu Ala Ala Phe Ala Cys Val Leu Leu Val
                405                 410                 415
Val Leu Phe Val Met Ile Asn Lys Tyr Gly Arg Arg Ser Lys Phe Gly
            420                 425                 430
Met Lys Gly Pro Val Ala Val Ile Ser Gly Glu Glu Asp Ser Ala Ser
        435                 440                 445
Pro Leu His His Ile Asn His Gly Ile Thr Thr Pro Ser Ser Leu Asp
    450                 455                 460
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Ala Gly Pro Asp Thr Val Val Ile Gly Met Thr Arg Ile Pro Val Ile
465                 470                 475                 480
Glu Asn Pro Gln Tyr Phe Arg Gln Gly His Asn Cys His Lys Pro Asp
                485                 490                 495
Thr Tyr Val Gln His Ile Lys Arg Arg Asp Ile Val Leu Lys Arg Glu
            500                 505                 510
Leu Gly Glu Gly Ala Phe Gly Lys Val Phe Leu Ala Glu Cys Tyr Asn
        515                 520                 525
Leu Ser Pro Thr Lys Asp Lys Met Leu Val Ala Val Lys Ala Leu Lys
    530                 535                 540
Asp Pro Thr Leu Ala Ala Arg Lys Asp Phe Gln Arg Glu Ala Glu Leu
545                 550                 555                 560
Leu Thr Asn Leu Gln His Glu His Ile Val Lys Phe Tyr Gly Val Cys
                565                 570                 575
Gly Asp Gly Asp Pro Leu Ile Met Val Phe Glu Tyr Met Lys His Gly
            580                 585                 590
Asp Leu Asn Lys Phe Leu Arg Ala His Gly Pro Asp Ala Met Ile Leu
        595                 600                 605
Val Asp Gly Gln Pro Arg Gln Ala Lys Gly Glu Leu Gly Leu Ser Gln
    610                 615                 620
Met Leu His Ile Ala Ser Gln Ile Ala Ser Gly Met Val Tyr Leu Ala
625                 630                 635                 640
Ser Gln His Phe Val His Arg Asp Leu Ala Thr Arg Asn Cys Leu Val
                645                 650                 655
Gly Ala Asn Leu Leu Val Lys Ile Gly Asp Phe Gly Met Ser Arg Asp
            660                 665                 670
Val Tyr Ser Thr Asp Tyr Tyr Arg Leu Phe Asn Pro Ser Gly Asn Asp
        675                 680                 685
Phe Cys Ile Trp Cys Glu Val Gly Gly His Thr Met Leu Pro Ile Arg
    690                 695                 700
Trp Met Pro Pro Glu Ser Ile Met Tyr Arg Lys Phe Thr Thr Glu Ser
705                 710                 715                 720
Asp Val Trp Ser Phe Gly Val Ile Leu Trp Glu Ile Phe Thr Tyr Gly
                725                 730                 735
Lys Gln Pro Trp Phe Gln Leu Ser Asn Thr Glu Val Ile Glu Cys Ile
            740                 745                 750
Thr Gln Gly Arg Val Leu Glu Arg Pro Arg Val Cys Pro Lys Glu Val
        755                 760                 765
Tyr Asp Val Met Leu Gly Cys Trp Gln Arg Glu Pro Gln Gln Arg Leu
    770                 775                 780
Asn Ile Lys Glu Ile Tyr Lys Ile Leu His Ala Leu Gly Lys Ala Thr
785                 790                 795                 800
Pro Ile Tyr Leu Asp Ile Leu Gly
                805
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<210> 57
<211> 2607
<212> DNA
<213> Homo sapiens

<400> 57
tgccctgcaa attgtgtctg cagcaagact gagatcaatt gccggcggcc ggacgatggg 60
aacctcttcc cctcctgga agggcaggat tcagggaaca gcaatgggaa cgccaatatc 120
aacatcaagg acatctcaag gaalatcact tccatacaca tagagaactg gcgcagtctt 180
cacacgctca acgccgtgga catggagctc tacaccggac ttcaaaagct gaccatcaag 240
aactcaggac ttcggagcat tcagccaaga gcctttgcca agaaacccca tttgcgttat 300
ataaacctgt caagtaaccg gatcaccaca atctcgtggc agcttttcca gacgctgagt 360

cttcgggaat tgcagttgga gcagaacttt ttcaactgca gctgtgacat ccgctggatg 420
cagctgtggc aggagcaggg ggaggccaag ctcaacagcc agaaccttca ctgcatcaat 480
gctgatggct cccagctccc tctcttccgc atgaacatca gtcagtgtga ccttcctgag 540
atcagcgtga gccacggtca cctgaccgta cgagagggtg ccactgctgt tatcacttgc 600
aatggctctg gatcaccctt tcctgatgtg gactggatag tcactgggct ggctgtcatc 660
aacaatctacc agaccaatct gaactggacc aatgttcatg ccatcaactt gacgcctggtg 720
aatgtgacga gtgaggacaa tggcttcacc ctgacgtgca ttgcagagga cgtggtgggc 780
atgagcaatg ccagtgttgc cctcactgtc tactatcccc cacgtgtggt gagcctggag 840
gagcctgagc tgcgcctgga gcactgcatc gagtttgtgg tgcgtggcaa ccccccacca 900
acgctgcact ggctgcacca tgggcagcct ctgcgggagt ccaagatcat ccatgtggaa 960
tactaccaag agggagagat ttccgagggc tgcctgctct tcaacaagcc cacccactac 1020
aacaatggca actatcccct cattgccaaa aacccactgg gcacagccaa ccagacatc 1080
aatggccact tcctcaagga ggcctttcca gaggagcacg atcacttcat cttgtttgac 1140
gaagtgagtc ccacacctcc tatcactgtg acccacaaac cagaagaaga cactttggg 1200
gtatccatag cagttggact tgctgctttt gcctgtgtcc tgttggtggt tctcttctgc 1260
atgatcaaca aatatggtcg acggtccaaa tttggaatga agggtcccgt ggctgtcatc 1320
agtggtgagg aggactcagc cagcccactg cacccatca accacggcat cacccacgccc 1380
tcgtcactgg atgccgggcc cgacacngtg gtcattggca tgactcgcat ccctgtcatt 1440
gagaacccac agtacttccg tcagggacac aactgccaca agccggacac gtatgtgcag 1500
cacattcaga ggagagacat cgtgctgaag cgagaactgg gtgagggagc ctttggaaag 1560
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aaggcctga aggatcccac cctggctgcc cggaaggatt tccagaggga ggccgagctg 1680
ctcaccaacc tgcagcatga gcacattgtc aagttctatg gagtgtgcgg cgatggggac 1740
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ctctttaatc catctggaaa tgatttttgt atatggtgtg aggtgggagg acacaccatg 2100
ctcccattc gctggatgcc tcctgaaagc atcatgtacc ggaagtttac tacagagagt 2160
gatgtatgga gcttcgggat gatcctctgg gagatcttca cctatggaaa gcagccatgg 2220
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cagcagcggt tgaacatcaa ggagatctac aaaatcctcc atgctttggg gaaggccacc 2400
ccaatctacc tggacattct tggctagtgg tggctggtgg tcatgaattc atactctgtt 2460
gcctcctctc tccctgcctc acatctccct tccacctcac aactccttcc atccttgact 2520
gaagcgaaca tcttcatata aactcaagtg cctgctacac atacaacact gaaaaaagga 2580
aaaaaaaaga aaaaaaaaaa aaaccgc 2607

<210> 58
<211> 360
<212> DNA
<213> Murine

<400> 58
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tcctgcaagt ctactggcta cacattcagt aacttcngga tagagtgggt aaagcagagg 120
cctggacatg gccttgagtg gattggagag attttacctg gcagtgatta tactaactac 180
aatgagaagt tcaaggccaa ggccacactc actgcagata catcctccaa cacagcctac 240
atgcaactca gcagcctgac atctgaggac tctgccgtct attactgtgc aagaaagaat 300
cgtaactact atggtaacta cgttgtatgg ggccaaggga ctctggtcac tgtctctgca 360

<210> 59
<211> 330
<212> DNA
<213> Murine

<400> 59
gacattgtga tgacccagtc tcctgcttcc ttagctgtat ctctggggca gagggccacc 60
atctcatacs gggccagcaa aagtgtcagt acatctggct atagtiatat gcactggaac 120
caacagaaac caggacagcc acccagactc ctcatctatc ttglatccaa cctagaatct 180
ggggtccctg ccaggttcag tggcagtggg tctgggacag acttcaccct caacatccat 240
cctgtggagg aggaggatgc tgcaacctat tactgtcagc acattaggga gcttacacgt 300
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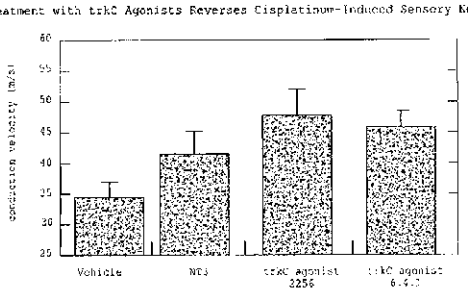
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(54) Title: AGONIST ANTI-TRK-C MONOCLONAL ANTIBODIES



(57) Abstract: The invention concerns agonist anti-trkC monoclonal antibodies which mimic certain biological activities of NT-3, the native ligand of trkC. The invention further concerns the use of such antibodies in the prevention and/or treatment of cellular degeneration, including nerve cell damage associated with acute nervous cell system injury and chronic neurodegenerative diseases, including peripheral neuropathy.

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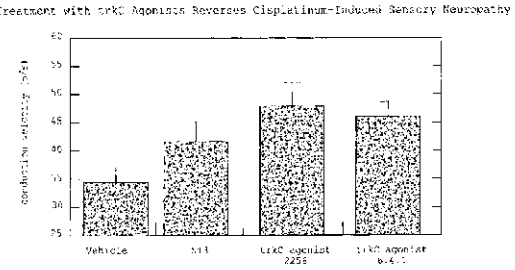
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[Continued on next page]

(54) Title: AGONIST ANTI TRK C MONOCLONAL ANTIBODIES



(57) Abstract: The invention concerns agonist anti-trkC monoclonal antibodies which mimic certain biological activities of NT-3, the native ligand of trkC. The invention further concerns the use of such antibodies in the prevention and/or treatment of cellular degeneration, including nerve cell damage associated with acute nervous cell system injury and chronic neurodegenerative diseases, including peripheral neuropathy.

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AGONIST ANTI-TRK-C MONOCLONAL ANTIBODIES

Background of the Invention

Field of the Invention

This invention concerns agonist anti-trkC monoclonal antibodies. It further concerns the use of the agonist antibodies in the prevention and/or treatment of cellular degeneration, including nerve cell damage associated with acute nervous cell system injury and chronic neurodegenerative diseases, including peripheral neuropathy.

Description of the Related Art

Neurotrophins are a family of small, basic proteins, which play a crucial role in the development and maintenance of the nervous system. The first identified and probably best understood member of this family is nerve growth factor (NGF), which has prominent effects on developing sensory and sympathetic neurons of the peripheral nervous system (Levi-Montalcini, R. and Angeletti, P.U., Physiol. Rev. 48, 534-569 [1968]; Thoenen, H. *et al.*, Rev. Physiol. Biochem. Pharmacol. 109, 145-178 [1987]). Although NGF had been known for a long time, including a homolog from the mouse submandibular gland, the mature, active form of which is often referred to as γ-or 2.5S NGF, it was only many years later that sequentially related but distinct polypeptides with similar functions were identified.

The first in line was a factor called *brain-derived* neurotrophic factor (BDNF), which was cloned and sequenced by Leibrock, J. *et al.* (Nature 341, 149-152 [1989]). This factor was originally purified from pig brain (Barde, Y.A. *et al.*, EMBO J. 1, 549-553 [1982]), but it was not until its cDNA was cloned and sequenced that its homology with NGF became apparent. The overall amino acid sequence identity between NGF and BNDF is about 50%. In view of this finding, Leibrock et al. speculated that there was no reason to think that BDNF and NGF should be the only members of a family of neurotrophins having in common structural and functional characteristics.

Indeed, further neurotrophins closely related to -NGF and BDNF have since been discovered. Several groups identified a neurotrophin originally called neuronal factor (NF), and now referred to as neurotrophin-3 (NT-3) (Ernfors *et al.*, Proc. Natl. Acad. Sci. USA 87, 5454-5458 [1990]; Höhn *et al.*, Nature 344, 339 [1990]; Maisonpierre *et al.*, Science 247, 1446 [1990]; Rosenthal *et al.*, Neuron 4, 767 [1990]; Jones and Reichardt, Proc. Natl. Acad. Sci. USA 87, 8060-8064 [1990]; Kaisho *et al.*, FEBS Lett. 266, 187 [1990]. NT-3 shares about 50% of its amino acids with both -NGF and BDNF (NT-2). Neurotrophins-4 and -5 (NT-4 and NT-5), have been added to the family (U.S. Patent No. 5,364,769 issued November 15, 1994; Hallbook, F. *et al.*, Neuron 6, 845-858 [1991]; Berkmeier, L.R. *et al.*, Neuron 7, 857-866 [1991]; Ip *et al.*, Proc. Natl. Acad. Sci USA 89, 3060-3064 [1992]). The mammalian molecule initially described by Berkmeier *et al.* supra, which was subsequently seen to be the homolog of Xenopus NT-4, is usually referred to as NT-4/5. In addition, there is an acidic homologous protein described in mammals which is referred to as NT-6 (Berkemeir, *et al.*, Somat. Cell Mol. Genet. 18(3):233-245 [1992]). More recently, another homologus protein from the fish, *Xiphophorus* has also been labeled NT-6 (Götz *et al.*, Nature 372:266-269 [1994]). There are two proteins described in the literature as NT-7, one cloned from the carp, *Cyprinus*, (Lai, *et al.*, Mol. Cell. Neurosci. 11(1-

2):64-76 [1998]] and one from the zebrafish, *Danio* (Nilsson *et al.*, FEBS Letters 424(3):235-90 [1998]). None of these last three described fish neurotrophins has been described outside fish, and their relationship to any mammalian neurotrophins is unclear. The amino acid sequence of zebrafish neurotrophin-7 (zNT-7) is more closely related to that of fish nerve growth factor (NGF) and neurotrophin-6 (NT-6) than to that of any other neurotrophin. zNT-7 is, however, equally related to fish NGF and NT-6 (65% and 63% amino acid sequence identity, respectively) indicating that it represents a distinct neurotrophin sequence. zNT-7 contains a 15 amino acid residue in a beta-turn region in the middle of the mature protein. Recombinant zNT-7 was able to bind to the human p75 neurotrophin receptor and to induce tyrosine phosphorylation of the rat trkA receptor tyrosine kinase, albeit less efficiently than rat NGF. zNT-7 did not interact with rat trkB or trkC, indicating a similar receptor specificity as NGF. We propose that a diversification of the NGF subfamily in the neurotrophin evolutionary tree occurred during the evolution of teleost fishes which in the appearance of several additional members, such as zNT-7 and NT-6, is structurally and functionally related to NGF.

Neurotrophins, similarly to other polypeptide growth factors, affect their target cells through interactions with cell surface receptors. According to our current knowledge, two kinds of transmembrane glycoproteins serve as receptors for neurotrophins. Equilibrium binding studies have shown that neurotrophin-responsive neurons possess a common low molecular weight (65-80 kDa), low affinity receptor (LNGFR), also termed as $p75^{NTR}$ or p75, which binds NGF, BDNF, and NT-3 with a K_d of 2×10^{-9} M, and large molecular weight (130-150 kDa), high affinity (K_d in the 10^{-11} M) receptors, which are members of the trk family of the receptor tyrosine kinases.

The first member of the trk receptor family, trkA, was initially identified as the result of an oncogenic transformation caused by the translocation of tropomyosin sequences onto its catalytic domain (Martin-Zanca *et al.*, Mol. Cell. Biol. 9(1):24-33 [1989]). Later work identified trkA as a signal transducing receptor for NGF. Subsequently, two other related receptors, mouse and rat trkB (Klein *et al.*, EMBO J. 8, 3701-3709 [1989]; Middlemas *et al.*, Mol. Cell. Biol, 11, 143-153 [1991]; EP 455,460 published 6 November 1991] and porcine, mouse and rat trkC (Lamballe *et al.*, Cell 66, 967-979 [1991]; EP 522,530 published 13 January 1993], were identified as members of the trk receptor family. The structures of the trk receptors are quite similar, but alternate splicing increases the complexity of the family by giving rise to two known forms of trkA, three known forms of trkB (two without functional tyrosine kinase domains) and at least four forms of trkC (several without functional tyrosine kinase domain, and two with small inserts in the tyrosine kinase domain).

The role of the p75 and trk receptors is controversial. It is generally accepted that trk receptor tyrosine kinases play an important role in conferring binding specificity to a particular neurotrophin, however, cell lines expressing trkA bind not only NGF but also NT-3 and NT-4/5 (but not BDNF), trkB expressing cells bind BDNF, NT-3, NT-4, and NT-4/5 (but not NGF), in contrast to trkC-expressing cells which have been reported to bind NT-3 alone (but not the other neurotrophins). Furthermore, it has been shown in model systems that the various forms of trk receptors, arising from alternate splicing events, can activate different intracellular signalling pathways, and therefore presumably mediate different physiological functions in vivo. It is unclear whether cells expressing a given trk receptor

in the absence of p75 bind neurotrophins with low or high affinity (Meakin and Shooter, Trends Neurosci. 15, 323-331 [1992]).

Published results of studies using various cell lines are confusing and suggest that p75 is either essential or dispensable for neurotrophin responsiveness. Cell lines that express p75 alone bind NGF, BDNF, NT-3, and NT-4 with similar low affinity at equilibrium, but the binding rate constants are remarkably different. As a result, although p75-binding is a common property of all neurotrophins, it has been suggested the p75 receptor may also play a role in ligand discrimination (Rodriguez-Tebar et al., EMBO J. 11, 917-922 [1992]). While the trk receptors have been traditionally thought of as the biologically significant neurotrophin receptors, it has recently been demonstrated that in melanoma cells devoid of trkA expression, NGF can still elicit profound changes in biological behavior presumably through p75 (Herrmann *et al.*, Mol. Biol. Cell 4, 1205-1216 [1993]). Davies *et al.* (Neuron 11, 565-574 [1993]) reported the results of studies investigating the role of p75 in mediating the survival response of embryonic neurons to neurotrophins in a model of transgenic mice carrying a null mutation in the p75 gene. They found that p75 enhances the sensitivity of NGF-dependent cutaneous sensory neurons to NGF. There have now been many studies showing that p75 is capable of mediating at least some of the biological effects of the neurotrophins. The field is still somewhat controversial, but p75 signaling has been implicated in controlling cell death, and neurite outgrowth. (Barker, PA, Cell Death Diff. 5:346-356 [1998]; Bredesen *et al.*, Cell Death Diff. 5:357-364 [1998]; Casaccia-Bonnefil, *et al.*, Cell Death Diff. 5:357-364 [1998]; Raoul *et al.*, Curr. Op. Neurobiol. 10:111-117 [2000]; Davies, AM, Curr. Biol. 10:R198-R200 [2000]). Importantly, stimulation of p75 has been shown to modify the effects of stimulating trkC (Hapner, *et al.*, Developm. Biol. 201:90-100 [1998]).

The extracellular domains of full-length native trkA, trkB and trkC receptors have five functional domains, that have been defined with reference to homologous or otherwise similar structures identified in various other proteins. The domains have been designated starting at the N-terminus of the amino acid sequence of the mature trk receptors as 1) a first cysteine-rich domain extending from amino acid position 1 to about amino acid position 32 of human trkA, from amino acid position 1 to about amino acid position 36 of human trkB, and from amino acid position 1 to about amino acid position 48 of human trkC; 2) a leucine-rich domain stretching from about amino acid 33 to about amino acid to about amino acid 104 in trkA; from about amino acid 37 to about amino acid 108 in trkB, and from about amino acid 49 to about amino acid 120 in trkC; 3) a second cysteine-rich domain from about amino acid 105 to about amino acid 157 in trkA; from about amino acid 109 to about amino acid 164 in trkB; and from about amino acid 121 to about amino acid 177 in trkC; 4) a first immunoglobulin-like domain stretching from about amino acid 178 to about amino acid 234 in trkA; from about amino acid 183 to about amino acid 239 in trkB; and from about amino acid 196 to about amino acid 257 in trkC; and 5) a second immunoglobulin-like domain extending from about amino acid 264 to about amino acid 330 in trkA; from about amino acid 270 to about amino acid 334 in trkB; and from about amino acid 288 to about amino acid 351 in trkC.

Neurotrophins exhibit actions on distinct, but overlapping, sets of peripheral and central neurons. These effects range from playing a crucial role in ensuring the survival of developing neurons (NGF in sensory and

sympathetic neurons) to relatively subtle effects on the *morphology of neurons* (NT-3 on purkinje cells). These activities have led to interest in using neurotrophins as treatments of certain neurodegenerative diseases. NT-3 has also been found to promote *proliferation* of peripheral *blood* leukocytes and, as a result, it has been suggested that NT-3 can be used in the treatment of neutropenia, infectious disease and tumors (U.S. Patent No. 6,015,552 issued on June 18, 2000).

The roles of neurotrophins in regulating cardiovascular development and modulating the vascular response to injury have also been investigated (Donovan *et al.*, Nature Genetics 14:210-213 [1996]; Donovan *et al.*, A.J. Path. 147:309-324 [1995]; Kraemer *et al.*, Arterial. Thromb. and Vasc. Biol. 19:1041-1050 [1999]). Neurotrophins have been described as potential therapeutics for regulating angiogenesis and vascular integrity (PCT Publication WO 00/24415, published May 4, 2000).

Despite their promise in the treatment of cellular degeneration, such as occurs due to neurodegenerative disease and acute neuronal injuries, and potentially angiogenesis, neurotrophins have several shortcomings. One significant shortcoming is the lack of specificity. Most neurotrophins cross-react with more than one receptor. For example NT-3, the preferred ligand of the trkC receptor tyrosine kinase, also binds to and activates trkA and trkB (Barbacid, J. Neurobiol. 25:1386-1403 [1994]; Barbacid, Ann. New York Acad. Sci. 766:442-458 [1995]; Ryden and Ibanez, J. Biol. Chem. 271:5623-5627 [1996]; Belliveau *et al.*, J. Cell. Biol. 136:375-388 [1997]; Farinas *et al.*, Neuron 21:325-334 [1998]). As a result, it is difficult to devise therapies that target a specific population of neurons. Another limitation of neurotrophin therapy is that neurotrophins, including NT-3 are known to elicit hyperalgesia (Chaudhry, *et al.*, Muscle and Nerve 23:189-192 [2000]). In addition, some neurotrophins such as NT-3 have poor pharmacokinetic and bioavailability properties in rodents, which raise serious questions about their human clinical applications (Haase *et al.*, J. Neurol. Sci. 160:S97-S105 [1998], dosages used in Helgren *et al.*, J. Neurosci. 17(1):372-82 [1997], and data below).

Accordingly, there is a great need for the development of new therapeutic agents for the treatment of neurodegenerative disorders and acute nerve cell injuries that are devoid of the known shortcomings of neurotrophins.

Summary of the Invention

The current invention is based on the development and characterization of agonist anti-trkC monoclonal antibodies, directed against epitopes in the extracellular domain of trkC receptor, which mimic the biological activities of NT-3, the natural ligand of trkC receptor but are free of some of the known detriments of NT-3. The invention also demonstrates the usefulness of these agonist antibodies in the treatment of neuropathy in an experimental animal model. Anti-trkC agonist antibodies offer numerous advantages over NT-3 in prophylactic or therapeutic treatment of cellular degeneration, such as nerve cell damage, in particular nerve cell injury associated with neurodegenerative diseases, such as peripheral neuropathies or due to external factors, such as trauma, toxic agents, surgery, just to mention a few.

In one aspect, the invention concerns an agonist anti-trkC monoclonal antibody which

(a) shows no significant cross-reactivity with trkA or trkB; and

(b) recognizes an epitope in domain 5 of trkC.

Certain agonist antibodies of the present invention may additionally recognize an epitope in domain 4 of trkC. In a preferred embodiment, the antibodies bind both human and rodent (e.g. rat or mouse) trkC, and may be murine, chimeric (including humanized) or human antibodies. The antibodies mimic at least one activity of the native trkC ligand, NT-3, and may thus be effective in the prevention and/or treatment of various diseases involving cellular degeneration, including, for example, neuropathies, such as cisplatin- or pyridoxine-induced neuropathy, or diabetic neuropathy, and (where cellular degeneration involves bone marrow cell degeneration) disorders of insufficient blood cells, such as leukopenias including eosinopenia and/or basopenia, lymphopenia, monocytopenia, and neutropenia. In a particularly preferred embodiment, the agonist antibodies of the present invention show superior properties over NT-3, for example, do not cause hyperalgesia when administered to a patient, have increased bioavailability and/or higher specific activity as compared to NT-3.

In another aspect, the invention concerns an anti-trkC antibody heavy chain comprising the following CDR's: a CDR1 selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4 and 5; a CDR2 selected from the group consisting of SEQ ID NOs: 6, 7, 8, 9, 10 and 11; and a CDR3 selected from the group consisting of SEQ ID NOs: 12, 13, 14, 15, 16 and 17.

In yet another aspect, the invention concerns an anti-trkC antibody light chain comprising the following CDR's: a CDR1 selected from the group consisting of SEQ ID NOs: 18, 19, 20, 21, 22, 23 and 24; a CDR2 selected from the group consisting of SEQ ID NOs: 25, 26, 27, 28, 29 and 30; and a CDR3 selected from the group consisting of SEQ ID NOs: 31, 32, 33, 34, 35 and 36.

In a further aspect, the invention concerns a murine anti-trkC antibody heavy chain comprising the following CDR's:

(a) a CDR1 of the formula XaaWXaaXaaWVK (SEQ ID NO: 37), wherein Xaa at position 1 is F or Y; Xaa at position 3 is I or M; and Xaa at position 4 is E or H;

(b) a CDR2 of the formula EIXaaPXaaXaaXaaXaaTNYNEKFKXaa (SEQ ID NO: 38), wherein Xaa at position 3 is L or Y; Xaa at position 5 is G or S; Xaa at position 6 is S or N; Xaa at position 7 is D or G; Xaa at position 8 is N or R and Xaa at position 16 is G or S; and

(c) a CDR3 of the formula KNRNYYGNYVV (SEQ ID NO: 12) or KYYYGNSYRSWYFDV (SEQ ID NO:13).

In a still further aspect, the invention relates to a human anti-trkC antibody heavy chain comprising the following CDR's:

(a) a CDR1 of the formula XaaXaaXaaYYWXaa (SEQ ID NO: 39), wherein Xaa at position 1 is S or I; Xaa at position 2 is G or S; Xaa at position 3 is G, T or Y, and Xaa at position 7 is S or N;

(b) a CDR2 of the formula XaaIXaaXaaSGSXaaTXaaNPSLKS (SEQ ID NO: 40), wherein Xaa at position 1 is Y or R; Xaa at position 3 is Y or F; Xaa at position 4 is Y or T; Xaa at position 8 is S or R; and Xaa at position 10 is N or Y; and

(c) a CDR3 of the formula selected from the group consisting of DRDYDSTGDYYSYYGMDV (SEQ ID NO: 14); DGGYSNPFD (SEQ ID NO: 15); ERIAAAGXaaDYYYNGLXaaV (SEQ ID NO: 41), wherein Xaa at position 8 is A or T and Xaa at position 16 is D or A.

In another aspect, the invention concerns an anti-trkC agonist monoclonal antibody comprising a heavy chain comprising the CDR's of the murine anti-trkC antibody heavy chain of claim 14 associated with a light chain. The antibody preferably is human or comprises human framework residues, and preferably shows no significant cross-reactivity with trkA or trkB. Throughout the application, antibodies are defined in the broadest sense, and specifically include antibody fragment, such as an Fv fragment, Fab fragment, Fab' or $F(ab')_2$ fragment. Antibodies of all classes and isotypes are included, but IgG, in particular IgG-2 and IgG-4 are preferred.

In yet another aspect, the invention concerns isolated nucleic acid encoding a murine or human anti-trkC agonist antibody heavy or light chain, or a fragment thereof. In a specific embodiment, the nucleic acid is a nucleic acid molecule deposited with ATCC on June 21, 2000 under an accession number selected from the group consisting of PTA-2133, PTA-2134, PTA-2135, PTA-2136, PTA-2137, PTA-2138, PTA-2139, PTA-2140, PTA-2141, PTA-2142 and PTA-2143.

In a further aspect, the invention concerns a vector comprising a nucleic acid molecule encoding an antibody heavy and/or light chain as hereinabove defined. The invention also concerns cells transformed with such nucleic acid. The invention further concerns hybridoma cell lines transformed with such nucleic acid and antibodies produced by such hybridoma cells.

In a still further aspect, the invention concerns a pharmaceutical composition comprising an effective amount of an agonist anti-trkC monoclonal antibody as hereinabove defined in admixture with a pharmaceutically acceptable carrier.

In another aspect, the invention concerns a method for treating a disease or condition involving cell degeneration, comprising administering to a mammal an effective amount of an agonist anti-trkC antibody disclosed herein.

In yet another aspect, the invention concerns a method for treating a neuropathy or neurodegenerative disease, or repairing a damaged nerve cell comprising administering to a mammal an effective amount of an agonist anti-trkC antibody disclosed herein. The neuropathy may, for example, be a peripheral neuropathy, including, without limitation, diabetic neuropathy and large-fiber sensory neuropathies. The neurodegenerative disease may, for example, be amyotrophic lateral sclerosis (ALS), Alzheimer's disease, Parkinson's disease, Huntington's disease. The damaged neurons may be peripheral, such as sensory, e.g. dorsal root ganglia neurons, motor neurons, e.g. neurons from the spinal cord, or central neurons, and the injury may be due to a variety of external and internal factors, including trauma, exposure to neurotoxins, metabolic diseases, infectious agents, etc.

In a further aspect, the invention concerns a method for promoting the development, proliferation, maintenance or regeneration of peripheral neurons, comprising contacting such neurons with an effective amount of an antibody of the present invention.

In a still further aspect, the invention concerns a method for the treatment (including prevention) of a disease or condition involving cell degeneration in a mammalian subject by introducing nucleic acid encoding an anti-trkC antibody herein into a cell of such subject. The method (gene therapy) preferably concerns the treatment of a neuropathy or neurodegenerative disease, or reparation of a damaged nerve cell. Accordingly, the recipient cells preferably are nerve cells.

In yet another aspect, the invention concerns delivery vehicles containing genetic material (nucleic acid) encoding an anti-trkC antibody suitable for gene therapy use.

In an additional aspect, the invention concerns a method of inducing angiogenesis by delivering an anti-trkC antibody of the present invention in an amount effective to induce angiogenesis. The delivery specifically includes the administration of the antibodies and the delivery of nucleic acid encoding the antibodies (e.g. in gene therapy).

In yet another aspect, the invention concerns an isolated nucleic acid molecule encoding a murine or human anti-trkC agonist antibody heavy or light chain selected from the group consisting of SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65; SEQ ID NO: 66; SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70 and SEQ ID NO: 71. The present invention also concerns a polypeptide encoded by one or more of the isolated nucleic acid molecules.

In another aspect, the invention concerns a whole cell transformed with nucleic acid encoding murine or human anti-trkC agonist antibody heavy chain, light chain or both heavy and light chain.

Brief Description of the Drawings

Figures 1A-D show agonist activity of various human (A and C) and murine (B and D) monoclonal antibodies against trkC receptor demonstrated using KIRA (A and B) and PC12 neurite outgrowth assay (C and D). Protein A purified monoclonal antibodies were diluted to 27 μg/ml in KIRA stimulation buffer (F12/DMEM 50:50 containing 2% bovine serum albumin (BSA, Intergen Co., Purchase, NY) and 25 mM Hepes, 0.2 μm filtered). The monoclonal antibodies were then diluted 1:3 (8 dilutions total; concentrations ranged from 0.01-180 nM Mab) in stimulation media. 6D-transfected CHO cells (5×10^4 cells/well) were then stimulated with either NT-3 or Mab (dilutions assayed in duplicate) for 6 hours and the assay was completes as described in the examples (Fig. 1A, human Mabs; Fig. 1B, murine Mabs). The purified Mabs were assayed for agonist activity in the PC12 neurite outgrowth assay as described in the examples. Rat PC12 cells were transfected with full-length human trkC and the cells plated at a density of 1000 cells/well. Three days following transfection, the Mabs were added in triplicate (concentrations ranging from 0.0002 to 2.7 nM) to the wells containing the trkC transfectants and incubated for an additional 3 days at 37 °C. The cells were then analyzed by phase contrast microscopy and cells with neurites exceeding two-times the diameter of the cell were counted.

Figure 2 shows that agonist anti-trkC monoclonal antibodies bind specifically to trkC using 8.1.2 antibody as a representative example.

Figure 3 demonstrates that agonist anti-trkC monoclonal antibodies recognize human trkC more efficiently than rat trkC. The ability of the monoclonal antibodies to bind rat trkC was determined using an immunoadhesin construct of the receptor. TrkC (human trkC-gD or rat trkC-IgG) was immobilized on microtiter plates (100 µl of a 1 µg/ml solution diluted in 50 mM carbonate buffer, pH 9.5) overnight. The plates were washed and blocked. The Mabs were then diluted to 1 µg/ml in PBS containing 0.5% BSA and 0.05% Tween 20, added to the appropriate wells (100 µl/well), and incubated for one hour at room temperature. The plates were washed and the appropriate HRP conjugate was added (human Mabs: goat anti-human κ-HRP, 1:5K; murine Mabs: goat anti-molgG (Fc)-HRP, 1:5 K) and incubated for one hour at room temperature. The plates were then washed, developed and read.

Figure 4 shows a representative example of epitope mapping using competition ELISA. A biotinylated human anti-trkC 6.1.2 monoclonal antibody was incubated with immobilized trkC in the absence or presence of excess of various unlabeled anti-trkC monoclonal antibodies.

Figure 5 summarizes the results of epitope mapping using competition ELISA.

Figures 6A-C show a schematic diagram of various trkC chimera (A) and their use in mapping of epitopes on trkC recognized by various agonist human (B) and murine (C) anti-trkC monoclonal antibodies.

Figure 7 shows amino acid sequence of human trkC domain 4 and 5 showing residues that were targeted for mutagenesis to decipher their roles in recognition by agonist anti-trkC monoclonal antibodies.

Figure 8 shows 3-dimensional ribbon diagram of trkC in complex with anti-trkC monoclonal antibodies. Specifically shown are the amino acid residues of trkC that are likely to play an important role in recognition by CDRs of anti-trkC antibodies.

Figure 9 shows the amino acid sequence of the heavy chain variable (V_H) region from murine and human anti-trkC agonist monoclonal antibodies. In addition, the three CDR regions (CDR1, CDR2 and CDR3) are highlighted in bold. The amino acid sequence of CDR1 of the 2250 and 2253 heavy chain is SEQ ID NO: 1. The amino acid sequence of CDR1 of the 2256 heavy chain is SEQ ID NO: 2. The amino acid sequence of CDR1 of the 6.1.2 and 2345 heavy chain is SEQ ID NO: 3. The amino acid sequence of CDR1 of the 6.4.1 heavy chain is SEQ ID NO: 4. The amino acid sequence of CDR1 of the 2349 heavy chain is SEQ ID NO: 5. The amino acid sequence of CDR2 of the 2250 and 2253 heavy chain is SEQ ID NO: 6. The amino acid sequence of CDR2 of the 2256 heavy chain is SEQ ID NO: 7. The amino acid sequence of CDR2 of the 6.1.2 heavy chain is SEQ ID NO: 8. The amino acid sequence of CDR2 of the 6.4.1 heavy chain is SEQ ID NO: 9. The amino acid sequence of CDR2 of the 2345 heavy chain is SEQ ID NO: 10. The amino acid sequence of CDR2 of the 2349 heavy chain is SEQ ID NO: 11. The amino acid sequence of CDR3 of the 2250 and 2253 heavy chain is SEQ ID NO: 12. The amino acid sequence of CDR3 of the 2256 heavy chain is SEQ ID NO: 13. The amino acid sequence of CDR3 of the 6.1.2 heavy chain is SEQ ID NO: 14. The amino acid sequence of CDR3 of the 6.4.1 heavy chain is SEQ ID NO: 15. The amino acid sequence of CDR3 of the 2345 heavy chain is SEQ ID NO: 16. The amino acid sequence of CDR3 of the 2349 heavy chain is SEQ ID NO: 17.

Figure 10 shows the amino acid sequence of the light chain variable (V_L) region from murine and human anti-trkC agonist monoclonal antibodies. In addition, the three CDR regions (CDR1, CDR2 and CDR3) are highlighted in bold. The amino acid sequence of CDR1 of the 2250 light chain is SEQ ID NO: 18. The amino acid sequence of CDR1 of the 2253 light chain is SEQ ID NO: 19. The amino acid sequence of CDR1 of the 2256 light chain is SEQ ID NO: 20. The amino acid sequence of CDR1 of the 6.1.2 light chain is SEQ ID NO: 21. The amino acid sequence of CDR1 of the 6.4.1 light chain is SEQ ID NO: 22. The amino acid sequence of CDR1 of the 2345 light chain is SEQ ID NO: 23. The amino acid sequence of CDR1 of the 2349 light chain is SEQ ID NO: 24. The amino acid sequence of CDR2 of the 2250 light chain is SEQ ID NO: 25. The amino acid sequence of CDR2 of the 2253 light chain is SEQ ID NO: 26. The amino acid sequence of CDR2 of the 2256 light chain is SEQ ID NO: 27. The amino acid sequence of CDR2 of the 6.1.2 light chain is SEQ ID NO: 28. The amino acid sequence of CDR2 of the 6.4.1 light chain is SEQ ID NO: 29. The amino acid sequence of CDR2 of the 2345 and 2349 light chain is SEQ ID NO: 30. The amino acid sequence of CDR3 of the 2250 light chain is SEQ ID NO: 31. The amino acid sequence of CDR3 of the 2253 light chain is SEQ ID NO: 32. The amino acid sequence of CDR3 of the 2256 light chain is SEQ ID NO: 33. The amino acid sequence of CDR3 of the 6.1.2 light chain is SEQ ID NO: 34. The amino acid sequence of CDR3 of the 6.4.1 light chain is SEQ ID NO: 35. The amino acid sequence of CDR3 of the 2345 and 2349 light chain is SEQ ID NO: 36.

Figure 11 shows amino acid sequence of CDRs of heavy and light variable chains of murine and human anti-trkC agonist monoclonal antibodies. Also shown are the families to which these sequences belong based on homology with CDR sequences available in databases.

Figure 12 shows that anti-trkC agonist monoclonal antibodies have improved half-life and bioavailability *in vivo*.

Figure 13 shows effect of anti-trkC agonist monoclonal antibodies on cisplatin-induced neuropathy.

Figure 14 shows decrease in marker expression caused by pyridoxine neuropathy.

Figure 15 shows amelioration of the effects of low doses of pyridoxine by agonist anti-trkC monoclonal antibodies.

Figure 16 shows amelioration of the effects of high doses of pyridoxine by agonist anti-trkC monoclonal antibodies.

Figure 17 shows amelioration of pyridoxine neuropathy by an anti-trkC agonist monoclonal antibody.

Figure 18 shows attenuation of pyridoxine-induced deficit of ladder by agonist anti-trkC monoclonal antibodies.

Figure 19 shows that NT3, but not anti-trkC agonist monoclonal antibodies, causes hyperalgesi at therapeutic doses.

Figure 20 shows the amino acid sequence of human trkC receptor (SEQ ID NO: 56) where the boundaries of domains 4 and 5 are indicated.

Figure 21 (in 2 pages) shows the nucleotide sequence of human trkC receptor (SEQ ID NO: 57).

Figure 22 shows the nucleotide sequence of the heavy chain (A; SEQ ID NO: 58) and light chain (B; SEQ ID NO: 59) of the anti-trkC agonist monoclonal antibody 2250.

Figure 23 shows the nucleotide sequence of the heavy chain (A; SEQ ID NO: 60) and light chain (B; SEQ ID NO: 61) of the anti-trkC agonist monoclonal antibody 2253.

Figure 24 shows the nucleotide sequence of the heavy chain (A; SEQ ID NO: 62) and light chain (B; SEQ ID NO: 63) of the anti-trkC agonist monoclonal antibody 2256.

Figure 25 shows the nucleotide sequence of the heavy chain (A; SEQ ID NO: 64) and light chain (B; SEQ ID NO: 65) of the anti-trkC agonist monoclonal antibody 2345.

Figure 26 shows the nucleotide sequence of the heavy chain (A; SEQ ID NO: 66) and light chain (B; SEQ ID NO: 67) of the anti-trkC agonist monoclonal antibody 2349.

Figure 27 shows the nucleotide sequence of the heavy chain (A; SEQ ID NO: 68) and light chain (B; SEQ ID NO: 69) of the anti-trkC agonist monoclonal antibody 6.1.2.

Figure 28 shows the nucleotide sequence of the heavy chain (A; SEQ ID NO: 70) and light chain (B; SEQ ID NO: 71) of the anti-trkC agonist monoclonal antibody 6.4.1.

Detailed Description of the Preferred Embodiment

A. Definitions

The term "neurotrophin" and its grammatical variants are used interchangeably, and refer to a family of polypeptides comprising nerve growth factor (NGF) and sequentially related homologs. NGF, brain-derived growth factor (BDNF, a.k.a. NT-2), neurotrophin-3 (NT-3), neurotrophins-4 and -5 (NT-4/5), neurotrophin-6 (NT-6), and neurotrophin-7 (NT-7) have so far been identified as members of this family.

The term "neurotrophin" includes native neurotrophins of any (human or non-human) animal species, and their functional derivatives, whether purified from a native source, prepared by methods of recombinant DNA technology, or chemical synthesis, or any combination of these or other methods. "Native" or "native sequence" neurotrophins have the amino acid sequence of a neurotrophin occurring in nature in any human or non-human animal species, including naturally-occurring truncated and variant forms, and naturally-occurring allelic variants.

The terms "trk", "trk polypeptide", "trk receptor" and their grammatical variants are used interchangeably and refer to polypeptides of the receptor tyrosine kinase superfamily, which are capable of binding at least one native neurotrophin. Currently identified members of this family are trkA ($gp140^{trkA}$), trkB, and trkC.

The expression "extracellular domain" or "ECD" when used herein refers to any polypeptide sequence that shares a ligand binding function of the extracellular domain of a naturally occurring receptor. Ligand binding function of the extracellular domain refers to the ability of the polypeptide to bind to a ligand. Accordingly, it is not necessary to include the entire extracellular domain since smaller segments have been found to be adequate for ligand binding. The truncated extracellular domain is generally soluble. The term ECD encompasses polypeptide sequences in which the hydrophobic transmembrane sequence (and, optionally, 1-20 amino acids C-terminal and/or N-terminal to the transmembrane domain) of the mature receptor has been deleted.

The term "agonist anti-trkC antibody" refers to an antibody, which is able to bind to and activate a native sequence trkC receptor and/or downstream pathways mediated by the trkC signaling function thereby mimicking a biological activity of a native ligand of the receptor, in particular NT-3. For example, the agonist antibody may bind to the ECD domain of a trkC receptor and thereby cause dimerization of the receptor, resulting in activation of the intracellular catalytic kinase domain. Consequently, this may result in stimulation of growth and/or differentiation of cells expressing the receptor *in vitro* and/or *in vivo*. The agonist antibodies of the present invention preferably recognize an epitope that includes at least part of domain 5 (amino acid positions from about 266 to about 381) and/or domain 4 (amino acid position from about 178 to about 265) of the human trkC receptor or a corresponding epitope on a non-human, e.g. murine trkC receptor.

"Biological activity", when used in conjunction with the agonist anti-trkC antibodies of the present invention, generally refers to having an effector function in common with NT-3, the native ligand of trkC. The effector function preferably is the ability to bind and activate the trkC receptor tyrosine kinase and/or downstream pathways mediated by the trkC signaling function. Without limitation, preferred biological activities include the ability to promote the development, proliferation, maintenance and/or regeneration of damaged cells, in particular neurons *in vitro* or *in vivo*,

including peripheral (sympathetic, parasympathetic, sensory, and enteric) neurons, motorneurons, and central (brain and spinal cord) neurons, and non-neuronal cells, e.g. peripheral blood leukocytes. A particularly preferred biological activity is the ability to treat (including prevention) a neuropathy, e.g. peripheral neuropathy or other neurodegenerative disease, or repair a damaged nerve cell. The damaged neurons may be sensory, sympathetic, parasympathetic, or enteric, e.g. dorsal root ganglia neurons, motorneurons, and central neurons, e.g. neurons from the spinal cord, and the damage may be of any cause, including trauma, toxic agents, surgery, stroke, ischemia, infection, metabolic disease, nutritional deficiency, and various malignancies. Another specific biological activity is the ability to induce angiogenesis.

As used herein, "treatment" is an approach for obtaining beneficial or desired clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. "Treatment" is an intervention performed with the intention of preventing the development or altering the pathology of a disorder. Accordingly, "treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented. Specifically, the treatment may directly prevent, slow down or otherwise decrease the pathology of cellular degeneration or damage, such as the pathology of nerve cells, or may render the cells, e.g. neurons more susceptible to treatment by other therapeutic agents. In a preferred embodiment, the treatment reduces or slows down the decline and/or stimulates the restoration of the function of target neurons.

The "pathology" of a (chronic) neurodegenerative disease or acute nervous system injury includes all phenomena that affect the well being of the patient including, without limitation, neuronal disfunction, degeneration, injury and/or death.

The terms "neurodegenerative disease" and "neurodegenerative disorder" are used in the broadest sense to include all disorders the pathology of which involves neuronal degeneration and/or disfunction, including, without limitation, peripheral neuropathies; motorneuron disorders, such as amyolotrophic lateral sclerosis (ALS, Lou Gehrig's disease), Bell's palsy, and various conditions involving spinal muscular atrophy or paralysis; and other human neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, epilepsy, multiple sclerosis, Huntington's chorea, Down's Syndrome, nerve deafness, and Meniere's disease.

"Peripheral neuropathy" is a neurodegenerative disorder that affects the peripheral nerves, most often manifested as one or a combination of motor, sensory, sensorimotor, or autonomic dysfunction. Peripheral neuropathies may, for example, be genetically acquired, can result from a systemic disease, or can be induced by a toxic agent, such as a neurotoxic drug, e.g. antineoplastic agent, or industrial or environmental pollutant. "Peripheral sensory neuropathy" is characterized by the degeneration of peripheral sensory neurons, which may be idiopathic, may occur, for example, as a consequence of diabetes (diabetic neuropathy), cytostatic drug therapy in cancer (e.g.

treatment with chemotherapeutic agents such as vincristine, cisplatin, methotrexate, 3'-azido-3'-deoxythymidine, or taxanes, *e.g.* paclitaxel [TAXOL®, Bristol-Myers Squibb Oncology, Princeton, NJ] and doxetaxel [TAXOTERE®, Rhône-Poulenc Rorer, Antony, France]), alcoholism, acquired immunodeficiency syndrom (AIDS), or genetic predisposition. Genetically acquired peripheral neuropathies include, for example, Refsum's disease, Krabbe's disease, Metachromatic leukodystrophy, Fabry's disease, Dejerine-Sottas syndrome, Abetalipoproteinemia, and Charcot-Marie-Tooth (CMT) Disease (also known as Proneal Muscular Atrophy or Hereditary Motor Sensory Neuropathy (HMSN)). Most types of peripheral neuropathy develop slowly, over the course of several months or years. In clinical practice such neuropathies are called chronic. Sometimes a peripheral neuropathy develops rapidly, over the course of a few days, and is referred to as acute. Peripheral neuropathy usually affects sensory and motor nerves together so as to cause a mixed sensory and motor neuropathy, but pure sensory and pure motor neuropathy are also known.

The term "toxic agent", as used in the context of the present invention, is meant to refer to a substance that, through its chemical action, injures, impairs, or inhibits the activity of a component of the nervous system. The long list of toxic agents (also referred to as "neurotoxic agents") includes, without limitation, chemotherapeutic agents, such as those listed above, alcohol, metals, industrial toxins, contaminants of food and medicines, etc.

"Mammal" for purpose of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sport or pet animals, such as dogs, horses, sheep, cats, cows, etc. Preferably, the mammal is human.

The term "trkC immunoadhesin" is used interchangeably with the expression "trkC-immunoglobulin chimera" and refers to a chimeric molecule that combines a portion of trkC (generally the extracellular domain thereof) with an immunoglobulin sequence. The immunoglobulin sequence preferably, but not necessarily, is an immunoglobulin constant domain. Chimeras constructed from a receptor sequence linked to an appropriate immunoglobulin constant domain sequence (immunoadhesins) are known in the art. Immunoadhesins reported in the literature include fusions of the T cell receptor* (Gascoigne *et al., Proc. Natl. Acad. Sci. USA*, 84: 2936-2940 [1987]); CD4* (Capon *et al., Nature* 337: 525-531 [1989]; Traunecker *et al., Nature*, 339: 68-70 [1989]; Zettmeissl *et al., DNA Cell Biol.* 9: 347-353 [1990]; Byrn *et al., Nature*, 344: 667-670 [1990]); L-selectin (homing receptor) (Watson *et al., J. Cell. Biol.*, 110:2221-2229 [1990]; Watson *et al., Nature*, 349: 164-167 [1991]); CD44* (Aruffo *et al., Cell*, 61: 1303-1313 [1990]); CD28* and B7* (Linsley *et al., J. Exp. Med.*, 173: 721-730 [1991]); CTLA-4* (Lisley *et al., J. Exp. Med.* 174: 561-569 [1991]); CD22* (Stamenkovic *et al., Cell*, 66:1133-11144 [1991]); TNF receptor (Ashkenazi *et al., Proc. Natl. Acad. Sci. USA*, 88: 10535-10539 [1991]; Lesslauer *et al., Eur. J. Immunol.*, 27:2883-2886 [1991]; Peppel *et al., J. Exp. Med.*, 174:1483-1489 [1991]); NP receptors (Bennett *et al., J. Biol. Chem.* 266:23060-23067 [1991]); and IgE receptor α* (Ridgway *et al., J. Cell. Biol.*, 115:abstr. 1448 [1991]), where the asterisk (*) indicates that the receptor is member of the immunoglobulin superfamily.

"Isolated" nucleic acid or polypeptide in the context of the present invention is a nucleic acid or polypeptide that is identified and separated from contaminant nucleic acids or polypeptides present in the animal or human source

of the nucleic acid or polypeptide. The nucleic acid or polypeptide may be labeled for diagnostic or probe purposes, using a label as described and defined further below in discussion of diagnostic assays.

In general, the term "amino acid sequence variant" refers to molecules with some differences in their amino acid sequences as compared to a reference (e.g. native sequence) polypeptide. The amino acid alterations may be substitutions, insertions, deletions or any desired combinations of such changes in a native amino acid sequence.

The terms "DNA sequence encoding", "DNA encoding" and "nucleic acid encoding" refer to the order or sequence of deoxyribonucleotides along a strand of deoxyribonucleic acid. The order of these deoxyribonucleotides determines the order of amino acids along the polypeptide chain. The DNA sequence thus codes for the amino acid sequence.

The terms "replicable expression vector" and "expression vector" refer to a piece of DNA, usually double-stranded, which may have inserted into it a piece of foreign DNA. Foreign DNA is defined as heterologous DNA, which is DNA not naturally found in the host cell. The vector is used to transport the foreign or heterologous DNA into a suitable host cell. Once in the host cell, the vector can replicate independently of the host chromosomal DNA, and several copies of the vector and its inserted (foreign) DNA may be generated. In addition, the vector contains the necessary elements that permit translating the foreign DNA into a polypeptide. Many molecules of the polypeptide encoded by the foreign DNA can thus be rapidly synthesized.

The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, a ribosome binding site, and possibly, other as yet poorly understood sequences. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancer.

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or a secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, then synthetic oligonucleotide adaptors or linkers are used in accord with conventional practice.

In the context of the present invention the expressions "cell", "cell line", and "cell culture" are used interchangeably, and all such designations include progeny. Thus, the words "transformants" and "transformed (host) cells" include the primary subject cell and cultures derived therefrom without regard for the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Mutant progeny that have the same function or biological activity as screened for in the originally transformed cell are included. Where distinct designations are intended, it will be clear from the context.

An "exogenous" element is defined herein to mean nucleic acid sequence that is foreign to the cell, or homologous to the cell but in a position within the host cell nucleic acid in which the element is ordinarily not found.

"Antibodies" (Abs) and "immunoglobulins" (Ig) are glycoproteins having the same structural characteristics. While antibodies exhibit binding specificity to a specific antigen, immunoglobulins include both antibodies and other antibody-like molecules that lack antigen specificity. Polypeptides of the latter kind are, for example, produced at low levels by the lymph system and at increased levels by myelomas.

"Native antibodies" and "native immunoglobulins" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light- chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light- and heavy-chain variable domains.

The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called hypervariable regions both in the light chain and the heavy chain variable domains. The more highly conserved portions of variable domains are called the framework region (FR). The variable domains of native heavy and light chains each comprise four FRs (FR1, FR2, FR3 and FR4, respectively), largely adopting a -sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the -sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat *et al., Sequences of Proteins of Immunological Interest,* 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991), pages 647-669). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

The term "hypervariable region" when used herein refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region comprises amino acid residues from a "complementarity determining region" or "CDR" (*i.e.* residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain; Kabat *et al., Sequences of Proteins of Immunological Interest,* 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)] and/or those residues from a "hypervariable loop" (*i.e.* residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain; Chothia and Lesk *J.*

Mol. Biol. 196:901-917 (1987)). "Framework" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined.

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

"Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This region consists of a dimer of one heavy chain and one light chain variable domain in tight, non-covalent association. It is in this configuration that the three hypervariable regions of each variable domain interact to define an antigen-binding site on the surface of the V_H-V_L dimer. Collectively, the six hypervariable regions confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three hypervariable regions specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

The Fab fragment also contains the constant domain of the light chain and the first constant domain (C_H1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxyl terminus of the heavy chain C_H1 domain including one or more cysteine(s) from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa ( ) and lambda ( ), based on the amino acid sequences of their constant domains.

Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. The heavy-chain constant domains that correspond to the different classes of immunoglobulins are called , , , , and , respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

The term "antibody" herein is used in the broadest sense and specifically covers human, non-human (e.g. murine) and humanized monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), and antibody fragments so long as they exhibit the desired biological activity.

"Antibody fragments" comprise a portion of a full length antibody, generally the antigen binding or variable domain thereof. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler *et al., Nature* 256:495 (1975), or may be made by recombinant DNA methods (see, *e.g.*, U.S. Patent No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson *et al., Nature* 352:624-628 (1991) and Marks *et al., J. Mol. Biol.* 222:581-597 (1991), for example.

The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; and Morrison *et al., Proc. Natl. Acad. Sci. USA* 81:6851-6855 (1984)).

"Humanized" forms of non-human (*e.g.*, murine) antibodies are chimeric antibodies which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which hypervariable region residues of the recipient are replaced by hypervariable region residues from a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues which are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable regions correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones *et al., Nature* 321:522-525 (1986); Reichmann *et al., Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992).

"Single-chain Fv" or "sFv" antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. Generally, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the sFv to form the desired structure for antigen binding. For a review

of sFv see Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds. Springer-Verlag, New York, pp. 269-315 (1994).

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy chain variable domain (V_H) connected to a light chain variable domain (V_L) in the same polypeptide chain (V_H - V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger *et al., Proc. Natl. Acad. Sci. USA* 90:6444-6448 (1993).

The expression "linear antibodies" when used throughout this application refers to the antibodies described in Zapata *et al. Protein Eng.* 8(10):1057-1062 (1995). Briefly, these antibodies comprise a pair of tandem Fd segments (V_H-C_H1-V_H-C_H1) which form a pair of antigen binding regions. Linear antibodies can be bispecific or monospecific.

The term "epitope" is used to refer to binding sites for (monoclonal or polyclonal) antibodies on protein antigens.

Antibodies which bind to domain 5 and/or 4 within the amino acid sequence of native sequence human trkC, or to an equivalent epitope in a native sequence non-human trkC receptor, are identified by "epitope mapping." There are many methods known in the art for mapping and characterizing the location of epitopes on proteins, including solving the crystal structure of an antibody-antigen complex, competition assays, gene fragment expression assays, and synthetic peptide-based assays, as described, for example, in Chapter 11 of Harlow and Lane, Using Antibodies, a Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1999. A competition ELISA assay is specifically described in Example 1. According to the gene fragment expression assays, the open reading frame encoding the protein is fragmented either randomly or by specific genetic constructions and the reactivity of the expressed fragments of the protein with the antibody to be tested is determined. The gene fragments may, for example, be produced by PCR and then transcribed and translated into protein *in vitro*, in the presence of radioactive amino acids. The binding of the antibody to the radioactively labeled protein fragments is then determined by immunoprecipitation and gel electrophoresis. Certain epitopes can also be identified by using large libraries of random peptide sequences displayed on the surface of phage particles (phage libraries). Alternatively, a defined library of overlapping peptide fragments can be tested for binding to the test antibody in simple binding assays. The latter approach is suitable to define linear epitopes of about 5 to 15 amino acids.

An antibody binds "essentially the same epitope" as a reference antibody, when the two antibodies recognize identical or sterically overlapping epitopes. The most widely used and rapid methods for determining whether two epitopes bind to identical or sterically overlapping epitopes are competition assays, which can be configured in all number of different formats, using either labeled antigen or labeled antibody. Usually, the antigen is immobilized on a 96-well plate, and the ability of unlabeled antibodies to block the binding of labeled antibodies is measured using radioactive or enzyme labels. A competition ELISA assay is disclosed in Example 1.

The term amino acid or amino acid residue, as used herein, refers to naturally occurring L amino acids or to D amino acids as described further below with respect to variants. The commonly used one- and three-letter abbreviations for amino acids are used herein (Bruce Alberts et al., Molecular Biology of the Cell, Garland Publishing, Inc., New York (3d ed. 1994)).

Hybridization is preferably performed under "stringent conditions" which means (1) employing low ionic strength and high temperature for washing, for example, 0.015 sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50 C, or (2) employing during hybridization a denaturing agent, such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42 C. Another example is use of 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6/8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 g/ml), 0.1% SDS, and 10% dextran sulfate at 42 C, with washes at 42 C in 0.2 x SSC and 0.1% SDS.

B. Methods for carrying out the invention

The present invention concerns agonist human and non-human monoclonal antibodies (including humanized forms of the latter), which mimick certain biological properties of NT-3, the native ligand of the trkC receptor. General techniques for the production of murine and human anti-trkC antibodies are well known in the art and are described hereinbelow. Further details, including the selection of agonist antibodies, are provided in Example 1.

1. Antibody preparation

(i) Polyclonal antibodies

Methods of preparing polyclonal antibodies are known in the art. Polyclonal antibodies can be raised in a mammal, for example, by one or more injections of an immunizing agent and, if desired, an adjuvant. Typically, the immunizing agent and/or adjuvant will be injected in the mammal by multiple subcutaneous or intraperitoneal injections. It may be useful to conjugate the immunizing agent to a protein known to be immunogenic in the mammal being immunized, such as serum albumin, or soybean trypsin inhibitor. Examples of adjuvants which may be employed include Freund's complete adjuvant and MPL-TDM.

(ii) Monoclonal antibodies

Monoclonal antibodies may be made using the hybridoma method first described by Kohler *et al., Nature,* 256:495 (1975), or may be made by recombinant DNA methods (U.S. Patent No. 4,816,567).

In the hybridoma method, a mouse or other appropriate host animal, such as a hamster or macaque monkey, is immunized as hereinabove described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Alternatively, lymphocytes may be immunized *in vitro*. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103, [Academic Press, 1986]).

The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For

example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), conditions under which the growth of HGPRT-deficient cells is prevented.

Preferred myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these, preferred myeloma cell lines are murine myeloma lines, such as those derived from MOP-21 and M.C.-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, California USA, and SP-2 or X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Maryland USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, *J. Immunol.*, 133:3001 (1984); Brodeur *et al.*, *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63, Marcel Dekker, Inc., New York, (1987)).

Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA).

The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson *et al.*, *Anal. Biochem.*, 107:220 (1980).

After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the cells may be subcloned by limiting dilution procedures and grown by standard methods (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103 (Academic Press, 1986)). Suitable culture media for this purpose include, for example, DMEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown *in vivo* as ascites tumors in an animal.

The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the monoclonal antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also may be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences, Morrison, *et al.*, Proc. Nat. Acad. Sci. 81, 6851 (1984), or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. In that

manner, "chimeric" or "hybrid" antibodies are prepared that have the binding specificity of an anti-trk monoclonal antibody herein.

Typically such non-immunoglobulin polypeptides are substituted for the constant domains of an antibody of the invention, or they are substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for an trk receptor and another antigen-combining site having specificity for a different antigen.

Chimeric or hybrid antibodies also may be prepared in vitro using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate.

Recombinant production of antibodies will be described in more detail below.

(iii) Humanized antibodies

Generally, a humanized antibody has one or more amino acid residues introduced into it from a non-human source. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers [Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature, 332:323-327 (1988); Verhoeyen et al., Science, 239:1534-1536 (1988)], by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody.

Accordingly, such "humanized" antibodies are chimeric antibodies (Cabilly, supra), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

It is important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three dimensional models of the parental and humanized sequences. Three dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e. the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the consensus and import sequence so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding. For further details see U.S. application Serial No. 07/934,373 filed 21 August 192, which is a continuation-in-part of application Serial No. 07/715,272 filed 14 June 1991.

(iv) Human antibodies

Human monoclonal antibodies can be made by the hybridoma method. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described, for example, by Kozbor, J. Immunol. 133, 3001 (1984), and Brodeur, *et al.*, Monoclonal Antibody Production Techniques and Applications, pp.51-63 (Marcel Dekker, Inc., New York, 1987).

It is now possible to produce transgenic animals (e.g. mice) that are capable, upon immunization, of producing a repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, e.g. Jakobovits *et al.*, Proc. Natl. Acad. Sci. USA 90, 2551-255 (1993); Jakobovits *et al.*, Nature 362, 255-258 (1993).

Mendez *et al. (Nature Genetics* 15: 146-156 (1997)) have further improved the technology and have generated a line of transgenic mice designated as "Xenomouse II" that, when challenged with an antigen, generates high affinity fully human antibodies. This was achieved by germ-line integration of megabase human heavy chain and light chain loci into mice with deletion into endogenous J_H segment as described above. The Xenomouse II harbors 1,020 kb of human heavy chain locus containing approximately 66 V_H genes, complete D_H and J_H regions and three different constant regions (μ, δ and χ), and also harbors 800 kb of human κ locus containing 32 Vκ genes, Jκ segments and Cκ genes. The antibodies produced in these mice closely resemble that seen in humans in all respects, including gene rearrangement, assembly, and repertoire. The human antibodies are preferentially expressed over endogenous antibodies due to deletion in endogenous J_H segment that prevents gene rearrangement in the murine locus.

Alternatively, the phage display technology (McCafferty *et al.*, Nature 348, 552-553 [1990]) can be used to produce human antibodies and antibody fragments *in vitro*, from immunoglobulin variable [V] domain gene repertoires from unimmunized donors. According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimicks some of the properties of the B-cell. Phage display can be performed in a variety of formats; for their review see, e.g. Johnson, Kevin S. and Chiswell, David J., Current Opinion in Structural Biology 3, 564-571 (1993). Several sources of V-gene segments can be used for phage display. Clackson *et al.*, Nature 352, 624-628 (1991) isolated a diverse array of anti-oxazolone antibodies from a small random combinatorial library of V genes derived from the spleens of immunized mice. A repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Marks *et al.*, J. Mol. Biol. 222, 581-597 (1991), or Griffith *et al.*, EMBO J. 12, 725-734 (1993). In a natural immune response, antibody genes accumulate

mutations at a high rate (somatic hypermutation). Some of the changes introduced will confer higher affinity, and B cells displaying high-affinity surface immunoglobulin are preferentially replicated and differentiated during subsequent antigen challenge. This natural process can be mimicked by employing the technique known as "chain shuffling" (Marks *et al.*, Bio/Technol. 10, 779-783 (1992)). In this method, the affinity of "primary" human antibodies obtained by phage display can be improved by sequentially replacing the heavy and light chain V region genes with repertoires of naturally occurring variants (repertoires) of V domain genes obtained from unimmunized donors. This techniques allows the production of antibodies and antibody fragments with affinities in the nM range. A strategy for making very large phage antibody repertoires (also known as "the mother-of-all libraries") has been described by Waterhouse *et al.*, Nucl. Acids Res. 21, 2265-2266 (1993), and the isolation of a high affinity human antibody directly from such large phage library is reported by Griffith *et al.*, EMBO J. (1994), in press. Gene shuffling can also be used to derive human antibodies from rodent antibodies, where the human antibody has similar affinities and specificities to the starting rodent antibody. According to this method, which is also referred to as "epitope imprinting", the heavy or light chain V domain gene of rodent antibodies obtained by phage display technique is replaced with a repertoire of human V domain genes, creating rodent-human chimeras. Selection on antigen results in isolation of human variable capable of restoring a functional antigen-binding site, i.e. the epitope governs (imprints) the choice of partner. When the process is repeated in order to replace the remaining rodent V domain, a human antibody is obtained (see PCT patent application WO 93/06213, published 1 April 1993). Unlike traditional humanization of rodent antibodies by CDR grafting, this technique provides completely human antibodies, which have no framework or CDR residues of rodent origin.

(v) Bispecific antibodies

Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for the trkC receptor to provide an agonist antibody, the other one is for any other antigen, and preferably for another receptor or receptor subunit. For example, bispecific antibodies specifically binding a trkC receptor and a neurotrophin, or a trkC receptor and another trk receptor are within the scope of the present invention.

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the coexpression of two immunoglobulin heavy chain-light chain pairs, where the two heavy chains have different specificities (Millstein and Cuello, Nature 305, 537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in PCT application publication No. WO 93/08829 (published 13 May 1993), and in Traunecker *et al.*, EMBO 10, 3655-3659 (1991).

According to a different and more preferred approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion

preferably is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, CH2 and CH3 regions. It is preferred to have the first heavy chain constant region (CH1) containing the site necessary for light chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are cotransfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance. In a preferred embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in PCT Publication No. WO 94/04690, published on March 3, 1994.

For further details of generating bispecific antibodies see, for example, Suresh *et al.*, Methods in Enzymology 121, 210 (1986).

(vi) Heteroconjugate antibodies

Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Patent No. 4,676,980), and for treatment of HIV infection (PCT application publication Nos. WO 91/00360 and WO 92/200373; EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. Patent No. 4,676,980, along with a number of cross-linking techniques.

(vii)Antibody fragments

In certain embodiments, the anti-trkC antibody (including murine, human and humanized antibodies, and antibody variants) is an antibody fragment. Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, *e.g.*, Morimoto *et al.*, *J. Biochem. Biophys. Methods* 24:107-117 (1992) and Brennan *et al.*, *Science* 229:81 (1985)). However, these fragments can now be produced directly by recombinant host cells. For example, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form $F(ab')_2$ fragments (Carter *et al.*, *Bio/Technology* 10:163-167 (1992)). In another embodiment, the $F(ab')_2$ is formed using the leucine zipper GCN4 to promote assembly of the $F(ab')_2$ molecule. According to another approach, Fv, Fab or $F(ab')_2$ fragments can be isolated directly from recombinant host cell culture. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner.

(viii) Amino acid sequence variants of antibodies

Amino acid sequence variants of the anti-trkC antibodies are prepared by introducing appropriate nucleotide changes into the anti-trkC antibody DNA, or by peptide synthesis. Such variants include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the anti-trkC antibodies of the examples herein. Any combination of deletion, insertion, and substitution is made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes also may alter post-translational processes of the humanized or variant anti-trkC antibody, such as changing the number or position of glycosylation sites.

A useful method for identification of certain residues or regions of the anti-trkC antibody that are preferred locations for mutagenesis is called "alanine scanning mutagenesis," as described by Cunningham and Wells *Science*, 244:1081-1085 (1989). Here, a residue or group of target residues are identified (*e.g.*, charged residues such as arg, asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with trkC antigen. Those amino acid locations demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at, or for, the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation *per se* need not be predetermined. For example, to analyze the performance of a mutation at a given site, ala scanning or random mutagenesis is conducted at the target codon or region and the expressed anti-trkC antibody variants are screened for the desired activity.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an anti-trkC antibody with an N-terminal methionyl residue or the antibody fused to an epitope tag. Other insertional variants of the anti-trkC antibody molecule include the fusion to the N- or C-terminus of the anti-trkC antibody of an enzyme or a polypeptide which increases the serum half-life of the antibody (see below).

Another type of variant is an amino acid substitution variant. These variants have at least one amino acid residue in the anti-trkC antibody molecule removed and a different residue inserted in its place. The sites of greatest interest for substitutional mutagenesis include the hypervariable regions, but FR alterations are also contemplated. Conservative substitutions are shown in Table 1 under the heading of "preferred substitutions". If such substitutions result in a change in biological activity, then more substantial changes, denominated "exemplary substitutions" in Table 1, or as further described below in reference to amino acid classes, may be introduced and the products screened.

Table 1

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	val; leu; ile	val
Arg (R)	lys; gln; asn	lys
Asn (N)	gln; his; asp, lys; arg	gln
Asp (D)	glu; asn	glu
Cys (C)	ser; ala	ser
Gln (Q)	asn; glu	asn
Glu (E)	asp; gln	asp
Gly (G)	ala	ala
His (H)	asn; gln; lys; arg	arg
Ile (I)	leu; val; met; ala; phe; norleucine	leu
Leu (L)	norleucine; ile; val; met; ala; phe	ile
Lys (K)	arg; gln; asn	arg
Met (M)	leu; phe; ile	leu
Phe (F)	leu; val; ile; ala; tyr	tyr
Pro (P)	ala	ala
Ser (S)	thr	thr
Thr (T)	ser	ser
Trp (W)	tyr; phe	tyr
Tyr (Y)	trp; phe; thr; ser	phe

Val (V)	ile; leu; met; phe; ala; norleucine	leu

Substantial modifications in the biological properties of the antibody are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

(1) hydrophobic: norleucine, met, ala, val, leu, ile;

(2) neutral hydrophilic: cys, ser, thr;

(3) acidic: asp, glu;

(4) basic: asn, gln, his, lys, arg;

(5) residues that influence chain orientation: gly, pro; and

(6) aromatic: trp, tyr, phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

Any cysteine residue not involved in maintaining the proper conformation of the humanized or variant anti-trkC antibody also may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking. Conversely, cysteine bond(s) may be added to the antibody to improve its stability (particularly where the antibody is an antibody fragment such as an Fv fragment).

A particularly preferred type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (*e.g.* a humanized or human antibody). Generally, the resulting variant(s) selected for further development will have improved biological properties relative to the parent antibody from which they are generated. A convenient way for generating such substitutional variants is affinity maturation using phage display. Briefly, several hypervariable region sites (*e.g.* 6-7 sites) are mutated to generate all possible amino substitutions at each site. The antibody variants thus generated are displayed in a monovalent fashion from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed variants are then screened for their biological activity (*e.g.* binding affinity) as herein disclosed. In order to identify candidate hypervariable region sites for modification, alanine scanning mutagenesis can be performed to identify hypervariable region residues contributing significantly to antigen binding. Alternatively, or in addition, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the antibody and human trkC. Such contact residues and neighboring residues are candidates for substitution according to the techniques elaborated herein. Once such variants are generated, the panel of variants is subjected to screening as described herein and antibodies with superior properties in one or more relevant assays may be selected for further development.

(ix) Glycosylation variants of antibodies

Antibodies are glycosylated at conserved positions in their constant regions (Jefferis and Lund, Chem. Immunol. 65:111-128 [1997]; Wright and Morrison, TibTECH 15:26-32 [1997]). The oligosaccharide side chains of the immunoglobulins affect the protein's function (Boyd *et al.*, Mol. Immunol. 32:1311-1318 [1996]; Wittwe and Howard, Biochem. 29:4175-4180 [1990]), and the intramolecular interaction between portions of the glycoprotein which can affect the conformation and presented three-dimensional surface of the glycoprotein (Hefferis and Lund, *supra*; Wyss and Wagner, Current Opin. Biotech. 7:409-416 [1996]). Oligosaccharides may also serve to target a given glycoprotein to certain molecules based upon specific recognition structures. For example, it has been reported that in agalactosylated IgG, the oligosaccharide moiety 'flips' out of the inter-CH2 space and terminal N-acetylglucosamine residues become available to bind mannose binding protein (Malhotra *et al.*, Nature Med. 1:237-243 [1995]). Removal by glycopeptidase of the oligosaccharides from CAMPATH-1H (a recombinant humanized murine monoclonal IgG1 antibody which recognizes the CDw52 antigen of human lymphocytes) produced in Chinese Hamster Ovary (CHO) cells resulted in a complete reduction in complement mediated lysis (CMCL) (Boyd *et al.*, Mol. Immunol. 32:1311-1318 [1996]), while selective removal of sialic acid residues using neuraminidase resulted in no loss of DMCL. Glycosylation of antibodies has also been reported to affect antibody-dependent cellular cytotoxicity (ADCC). In particular, CHO cells with tetracycline-regulated expression of β(1,4)-N-acetylglucosaminyltransferase III (GnTIII), a glycosyltransferase catalyzing formation of bisecting GlcNAc, was reported to have improved ADCC activity (Umana *et al.*, Mature Biotech. 17:176-180 [1999]).

Glycosylation variants of antibodies are variants in which the glycosylation pattern of an antibody is altered. By altering is meant deleting one or more carbohydrate moieties found in the antibody, adding one or more carbohydrate moieties to the antibody, changing the composition of glycosylation (glycosylation pattern), the extent of glycosylation, etc. Glycosylation variants may, for example, be prepared by removing, changing and/or adding one or more glycosylation sites in the nucleic acid sequence encoding the antibody.

Glycosylation of antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

Addition of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation sites).

Nucleic acid molecules encoding amino acid sequence variants of the anti-trkC antibody are prepared by a variety of methods known in the art. These methods include, but are not limited to, isolation from a natural source (in

the case of naturally occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (*or site*-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of the anti-trkC antibody.

The glycosylation (including glycosylation pattern) of antibodies may also be altered without altering the underlying nucleotide sequence. Glycosylation largely depends on the host cell used to express the antibody. Since the cell type used for expression of recombinant glycoproteins, e.g. antibodies, as potential therapeutics is rarely the native cell, significant variations in the glycosylation pattern of the antibodies can be expected (see, e.g. Hse *et al.*, J. Biol. Chem. 272:9062-9070 [1997]). In addition to the choice of host cells, factors which affect glycosylation during recombinant production of antibodies include growth mode, media formulation, culture density, oxygenation, pH, purification schemes and the like. Various methods have been proposed to alter the glycosylation pattern achieved in a particular host organism including introducing or overexpressing certain enzymes involved in oligosaccharide production (U. S. Patent Nos. 5,047,335; 5,510,261 and 5,278,299). Glycosylation, or certain types of glycosylation, can be enzymatically removed from the glycoprotein, for example using endoglycosidase H (Endo H). In addition, the recombinant host cell can be genetically engineered, e.g. make defective in processing certain types of polysaccharides. These and similar techniques are well known in the art.

The glycosylation structure of antibodies can be readily analyzed by conventional techniques of carbohydrate analysis, including lectin chromatography, NMR, Mass spectrometry, HPLC, GPC, monosaccharide compositional analysis, sequential enzymatic digestion, and HPAEC-PAD, which uses high pH anion exchange chromatography to separate oligosaccharides based on charge. Methods for releasing oligosaccharides for analytical purposes are also known, and include, without limitation, enzymatic treatment (commonly performed using peptide-N-glycosidase F/endo-β-galactosidase), elimination using harsh alkaline environment to release mainly O-linked structures, and chemical methods using anhydrous hydrazine to release both N- and O-linked oligosaccharides.

(x) Other modifications of antibodies

The anti-trkC antibodies disclosed herein may also be formulated as immunoliposomes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein *et al., Proc. Natl. Acad. Sci. USA* 82:3688 (1985); Hwang *et al., Proc. Natl Acad. Sci, USA* 77:4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present invention can be conjugated to the liposomes as described in Martin *et al., J. Biol. Chem.* 257:286-288 (1982) via a disulfide interchange reaction. A chemotherapeutic agent (such as Doxorubicin) is optionally contained within the liposome. See Gabizon *et al., J. National Cancer Inst.*81(19):1484 (1989).

The antibody of the present invention may also be used in ADEPT by conjugating the antibody to a prodrug-activating enzyme which converts a prodrug (*e.g.*, a peptidyl chemotherapeutic agent, see WO81/01145) to an active anti-cancer drug. See, for example, WO 88/07378 and U.S. Patent No. 4,975,278.

The enzyme component of the immunoconjugate useful for ADEPT includes any enzyme capable of acting on a prodrug in such a way so as to covert it into its more active, cytotoxic form.

Enzymes that are useful in the method of this invention include, but are not limited to, alkaline phosphatase useful for converting phosphate-containing prodrugs into free drugs; arylsulfatase useful for converting sulfate-containing prodrugs into free drugs; cytosine deaminase useful for converting non-toxic 5-fluorocytosine into the anti-cancer drug, 5-fluorouracil; proteases, such as serratia protease, thermolysin, subtilisin, carboxypeptidases and cathepsins (such as cathepsins B and L), that are useful for converting peptide-containing prodrugs into free drugs; D-alanylcarboxypeptidases, useful for converting prodrugs that contain D-amino acid substituents; carbohydrate-cleaving enzymes such as -galactosidase and neuraminidase useful for converting glycosylated prodrugs into free drugs; -lactamase useful for converting drugs derivatized with -lactams into free drugs; and penicillin amidases, such as penicillin V amidase or penicillin G amidase, useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups, respectively, into free drugs. Alternatively, antibodies with enzymatic activity, also known in the art as "abzymes", can be used to convert the prodrugs of the invention into free active drugs (see, *e.g.*, Massey, *Nature* 328:457-458 (1987)). Antibody-abzyme conjugates can be prepared as described herein for delivery of the abzyme to a tumor cell population.

The enzymes of this invention can be covalently bound to the anti-trkC antibodies by techniques well known in the art such as the use of the heterobifunctional crosslinking reagents discussed above. Alternatively, fusion proteins comprising at least the antigen binding region of an antibody of the invention linked to at least a functionally active portion of an enzyme of the invention can be constructed using recombinant DNA techniques well known in the art (see, *e.g.*, Neuberger *et al.*, *Nature* 312:604-608 (1984)).

In certain embodiments of the invention, it may be desirable to use an antibody fragment, rather than an intact antibody. In this case, it may be desirable to modify the antibody fragment in order to increase its serum half-life. This may be achieved, for example, by incorporation of a salvage receptor binding epitope into the antibody fragment (*e.g.*, by mutation of the appropriate region in the antibody fragment or by incorporating the epitope into a peptide tag that is then fused to the antibody fragment at either end or in the middle, *e.g.*, by DNA or peptide synthesis). See WO96/32478 published October 17, 1996.

The salvage receptor binding epitope generally constitutes a region wherein any one or more amino acid residues from one or two loops of a Fc domain are transferred to an analogous position of the antibody fragment. Even more preferably, three or more residues from one or two loops of the Fc domain are transferred. Still more preferred, the epitope is taken from the CH2 domain of the Fc region (*e.g.*, of an IgG) and transferred to the CH1, CH3, or V_H region, or more than one such region, of the antibody. Alternatively, the epitope is taken from the CH2 domain of the Fc region and transferred to the C_L region or V_L region, or both, of the antibody fragment.

Covalent modifications of the humanized or variant anti-trkC antibody (including glycosylation variants) are also included within the scope of this invention. They may be made by chemical synthesis or by enzymatic or chemical cleavage of the antibody, if applicable. Other types of covalent modifications of the antibody are introduced into the molecule by reacting targeted amino acid residues of the antibody with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues. Exemplary covalent modifications of polypeptides are described in US Patent 5,534,615, specifically incorporated herein by reference. A preferred type of covalent modification of the antibody comprises linking the antibody to one of a variety of nonproteinaceous polymers, *e.g.*, polyethylene glycol, polypropylene glycol, or polyoxyalkylenes, in the manner set forth in U.S. Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337.

2. Vectors, Host Cells and Recombinant Methods

The invention also provides isolated nucleic acid encoding the non-human, e.g. murine and human anti-trkC antibodies of the present invention (including the humanized versions of the non-human antibodies), vectors and host cells comprising the nucleic acid, and recombinant techniques for the production of the antibodies.

For recombinant production of an antibody, the nucleic acid encoding it may be isolated and inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. In another embodiment, the antibody may be produced by homologous recombination, *e.g.* as described in US Patent 5,204,244, specifically incorporated herein by reference. DNA encoding the monoclonal antibody is readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody). Many vectors are available. The vector components generally include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence, *e.g.*, as described in US Patent 5,534,615 issued July 9, 1996 and specifically incorporated herein by reference.

Suitable host cells for cloning or expressing the DNA in the vectors herein are the prokaryote, yeast, or higher eukaryote cells described above. Suitable prokaryotes for this purpose include eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as *Escherichia*, *e.g.*, *E. coli*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Proteus*, *Salmonella*, *e.g.*, *Salmonella typhimurium*, *Serratia*, *e.g.*, *Serratia marcescans*, and *Shigella*, as well as *Bacilli* such as *B. subtilis* and *B. licheniformis* (*e.g.*, *B. licheniformis* 41P disclosed in DD 266,710 published 12 April 1989), *Pseudomonas* such as *P. aeruginosa*, and *Streptomyces*. One preferred *E. coli* cloning host is *E. coli* 294 (ATCC 31,446), although other strains such as *E. coli* B, *E. coli* X1776 (ATCC 31,537), and *E. coli* W3110 (ATCC 27,325) are suitable. These examples are illustrative rather than limiting.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for anti-trkC antibody-encoding vectors. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *Schizosaccharomyces pombe*; *Kluyveromyces* hosts such as, *e.g.*, *K. lactis*, *K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC 16,045), *K. wickeramii* (ATCC 24,178), *K. waltii*

(ATCC 56,500), *K. drosophilarum* (ATCC 36,906), *K . thermotolerans*, and *K. marxianus; yarrowia* (EP 402,226); *Pichia pastoris* (EP 183,070); *Candida; Trichoderma reesia* (EP 244,234); *Neurospora crassa*; *Schwanniomyces* such as *Schwanniomyces occidentalis*; and filamentous fungi such as, *e.g., Neurospora, Penicillium, Tolypocladium*, and *Aspergillus* hosts such as *A. nidulans* and *A. niger*.

Suitable host cells for the expression of glycosylated anti-trkC antibody are derived from multicellular organisms. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda* (caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosophila melanogaster* (fruitfly), and *Bombyx mori* have been identified. A variety of viral strains for transfection are publicly available, *e.g.*, the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of *Spodoptera frugiperda* cells. Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, and tobacco can also be utilized as hosts.

However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al., J. Gen Virol.* 36:59 [1977]); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub *et al., Proc. Natl. Acad. Sci. USA* 77:4216 [1980]); mouse sertoli cells (TM4, Mather, *Biol. Reprod.* 23:243-251 [1980]); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HeLa, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather *et al., Annals N.Y. Acad. Sci.* 383:44-68 [1982]); MRC 5 cells; and FS4 cells.

Host cells are transformed with the above-described expression or cloning vectors for anti-trkC antibody production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

The host cells used to produce the anti-trkC antibody of this invention may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ((MEM) (Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium (DMEM) (Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham *et al., Meth. Enz.* 58:44 (1979), Barnes *et al., Anal. Biochem.* 102:255 (1980), U.S. Pat. Nos. 4,767,704; 4,657,866; 4,927,762; 4,560,655; or 5,122,469; WO 90/03430; WO 87/00195; or U.S. Patent Re. 30,985 may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleotides (such as adenosine and thymidine), antibiotics (such as GENTAMYCIN™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any

other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

When using recombinant techniques, the antibody can be produced intracellularly, in the periplasmic space, or directly secreted into the medium. If the antibody is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, is removed, for example, by centrifugation or ultrafiltration. Carter *et al.*, *Bio/Technology* 10:163-167 (1992) describe a procedure for isolating antibodies which are secreted to the periplasmic space of *E. coli*. Briefly, cell paste is thawed in the presence of sodium acetate (pH 3.5), EDTA, and phenylmethylsulfonylfluoride (PMSF) over about 30 min. Cell debris can be removed by centrifugation. Where the antibody is secreted into the medium, supernatants from such expression systems are generally first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. A protease inhibitor such as PMSF may be included in any of the foregoing steps to inhibit proteolysis and antibiotics may be included to prevent the growth of adventitious contaminants.

The antibody composition prepared from the cells can be purified using, for example, hydroxylapatite chromatography, gel electrophoresis, dialysis, and affinity chromatography, with affinity chromatography being the preferred purification technique. The suitability of protein A as an affinity ligand depends on the species and isotype of any immunoglobulin Fc domain that is present in the antibody. Protein A can be used to purify antibodies that are based on human 1, 2, or 4 heavy chains (Lindmark *et al., J. Immunol. Meth.* 62:1-13 (1983)). Protein G is recommended for all mouse isotypes and for human 3 (Guss *et al., EMBO J.* 5:15871575 (1986)). The matrix to which the affinity ligand is attached is most often agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrenedivinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Where the antibody comprises a C_H3 domain, the Bakerbond ABX™ resin (J. T. Baker, Phillipsburg, NJ) is useful for purification. Other techniques for protein purification such as fractionation on an ion-exchange column, ethanol precipitation, Reverse Phase HPLC, chromatography on silica, chromatography on heparin SEPHAROSE™ chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also available depending on the antibody to be recovered.

Following any preliminary purification step(s), the mixture comprising the antibody of interest and contaminants may be subjected to low pH hydrophobic interaction chromatography using an elution buffer at a pH between about 2.5-4.5, preferably performed at low salt concentrations (*e.g.*, from about 0-0.25M salt).

3. Identification of Agonist anti-trkC Antibodies

Agonist antibodies may be identified, for example, using the kinase receptor activation (KIRA) assay described in U. S. Patent Nos. 5,766,863 and 5,891,650. This ELISA-type assay is suitable for qualitative or quantitative measurement of kinase activation by measuring the autophosphorylation of the kinase domain of a receptor protein tyrosine kinase (rPTK, e.g. trk receptor), as well as for identification and characterization of potential

agonist or antagonists of a selected rPTK. The first stage of the assay involves phosphorylation of the kinase domain of a kinase receptor, in the present case a trkC receptor, wherein the receptor is present in the cell membrane of a eukaryotic cell. The receptor may be an endogenous receptor or nucleic acid encoding the receptor, or a receptor construct, may be transformed into the cell. Typically, a first solid phase (*e.g.*, a well of a first assay plate) is coated with a substantially homogeneous population of such cells (usually a mammalian cell line) so that the cells adhere to the solid phase. Often, the cells are adherent and thereby adhere naturally to the first solid phase. If a "receptor construct" is used, it usually comprises a fusion of a kinase receptor and a flag polypeptide. The flag polypeptide is recognized by the capture agent, often a capture antibody, in the ELISA part of the assay. An analyte, such as a candidate agonist, is then added to the wells having the adherent cells, such that the tyrosine kinase receptor (e.g. trkC receptor) is exposed to (or contacted with) the analyte. This assay enables identification of agonist ligands for the tyrosine kinase receptor of interest (e.g. trkC). It is also possible to use this assay to detect antagonists of a tyrosine kinase receptor. In order to detect the presence of an antagonist ligand which blocks binding of an agonist to the receptor, the adhering cells are exposed to the suspected antagonist ligand first, and then to the agonist ligand, so that competitive inhibition of receptor binding and activation can be measured. Also, the assay can identify an antagonist which binds to the agonist ligand and thereby reduces or eliminates its ability to bind to, and activate, the rPTK. To detect such an antagonist, the suspected antagonist and the agonist for the rPTK are incubated together and the adhering cells are then exposed to this mixture of ligands. Following exposure to the analyte, the adhering cells are solubilized using a lysis buffer (which has a solubilizing detergent therein) and gentle agitation, thereby releasing cell lysate which can be subjected to the ELISA part of the assay directly, without the need for concentration or clarification of the cell lysate.

The cell lysate thus prepared is then ready to be subjected to the ELISA stage of the assay. As a first step in the ELISA stage, a second solid phase (usually a well of an ELISA microtiter plate) is coated with a capture agent (often a capture antibody) which binds specifically to the tyrosine kinase receptor, or, in the case of a receptor construct, to the flag polypeptide. Coating of the second solid phase is carried out so that the capture agent adheres to the second solid phase. The capture agent is generally a monoclonal antibody, but, as is described in the examples herein, polyclonal antibodies may also be used. The cell lysate obtained is then exposed to, or contacted with, the adhering capture agent so that the receptor or receptor construct adheres to (or is captured in) the second solid phase. A washing step is then carried out, so as to remove unbound cell lysate, leaving the captured receptor or receptor construct. The adhering or captured receptor or receptor construct is then exposed to, or contacted with, an anti-phosphotyrosine antibody which identifies phosphorylated tyrosine residues in the tyrosine kinase receptor. In the preferred embodiment, the anti-phosphotyrosine antibody is conjugated (directly or indirectly) to an enzyme which catalyses a color change of a non-radioactive color reagent. Accordingly, phosphorylation of the receptor can be measured by a subsequent color change of the reagent. The enzyme can be bound to the anti-phosphotyrosine antibody directly, or a conjugating molecule (*e.g.*, biotin) can be conjugated to the anti-phosphotyrosine antibody and the enzyme can be subsequently bound to the anti-phosphotyrosine antibody via the conjugating molecule. Finally,

binding of the anti-phosphotyrosine antibody to the captured receptor or receptor construct is measured, *e.g.*, by a color change in the color reagent.

Following initial identification, the agonist activity can be further confirmed and refined by bioassays, known to test the targeted biological activities. For example, the ability of anti-trkC monoclonal antibodies to mimic the activity of NT-3 can be tested in the PC12 neurite outgrowth assay as described in Example 1, and confirmed in known animal models of neurodegenerative diseases, such as the experimental animal models of cisplatin- and pyridoxine-induced neuropathies described in Example 2.

3. Therapeutic and Diagnostic Uses of Agonist anti-TrkC Antibodies

The anti-trkC *agonist antibodies* of the present invention are believed to be useful in the treatment (including prevention) of disorders the pathology of which involves cellular degeneration or disfunction. In particular, the anti-trkC agonist antibodies are promising candidates for the treatment of various (chronic) neurodegenerative disorders and acute nerve cell injuries. Such neurodegenerative disorders include, without limitation, peripheral neuropathies; motorneuron disorders, such as amyotrophic lateral sclerosis (ALS, Lou Gehrig's disease), Bell's palsy, and various conditions involving spinal muscular atrophy or paralysis; and other human neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, epilepsy, multiple sclerosis, Huntington's chorea, Down's Syndrome, nerve deafness, and Meniere's disease, and acute nerve cell injuries, for example due to trauma or spinal cord injury.

The anti-trkC antibodies of the present invention are believed to be particularly suited for the treatment of peripheral neuropathy, a neurodegenerative disorder that affects the peripheral nerves, most often manifested as one or a combination of motor, sensory, sensorimotor, or autonomic dysfunction. Peripheral neuropathies may, for example, be genetically acquired, can result from a systemic disease, can be induced by a toxic agent, such as a neurotoxic drug, e.g. antineoplastic agent, or industrial or environmental pollutant, or can be idiopathic. Thus, peripheral sensory neuropathy is characterized by the degeneration, decrease or failure of function of peripheral sensory neurons, which may occur, for example, as a consequence of diabetes (diabetic neuropathy), cytostatic drug therapy in cancer (e.g. treatment with chemotherapeutic agents such as vincristine, cisplatin, methotrexate, or 3'-azido-3'-deoxythymidine), alcoholism, acquired immunodeficiency syndrome (AIDS), or genetic predisposition. Genetically acquired peripheral neuropathies include, for example, Refsum's disease, Krabbe's disease, Metachromatic leukodystrophy, Fabry's disease, Dejerine-Sottas syndrome, Abetalipoproteinemia, and Charcot-Marie-Tooth (CMT) Disease (also known as Proneal Muscular Atrophy or Hereditary Motor Sensory Neuropathy (HMSN)).

Based on the demonstrated ability of NT-3, the native ligand of the trkC receptor, to promote proliferation of peripheral blood leukocytes, the anti-trkC agonist antibodies of the present invention may be used also as therapeutic agents for the treatment of neutropenia, various infections, and tumors. Since the expression of trkC is not limited to neurons, anti-trkC agonist antibodies are expected to find utility in the prevention or treatment of disorders characterized by cellular degeneration in general, without restriction to neural cells.

The anti-trkC antibodies of the present invention may also be used to *induce angiogenesis, or treat* pathological conditions/diseases in which the induction of angiogenesis is desirable. Such pathological conditions include, for example, cardiac ischemia regardless of the underlying pathology, including cerebrovascular disorders caused by insufficient cerebral circulation. Angiogenesis may also be desirable in the treatment of wounds, including ulcers, diabetic complications of sickle cell disease, and post surgical wounds.

The anti-trkC antibodies of the present invention may also be useful in the diagnosis of diseases involving cellular degeneration, in particular the neurodegenerative diseases listed above.

For diagnostic applications, the antibody typically will be labeled with a detectable moiety. Numerous labels are available which can be generally grouped into the following categories:

(a) Radioisotopes, such as ^{35}S, ^{14}C, ^{125}I, ^{3}H, and ^{131}I. The antibody can be labeled with the radioisotope using the techniques described in *Current Protocols in Immunology*, Volumes 1 and 2, Coligen *et al.*, Ed. Wiley-Interscience, New York, New York, Pubs. (1991) for example and radioactivity can be measured using scintillation counting.

(b) Fluorescent labels such as rare earth chelates (europium chelates) or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, Lissamine, phycoerythrin and Texas Red are available. The fluorescent labels can be conjugated to the antibody using the techniques disclosed in *Current Protocols in Immunology, supra*, for example. Fluorescence can be quantified using a fluorimeter.

(c) Various enzyme-substrate labels are available and U.S. Patent No. 4,275,149 provides a review of some of these. The enzyme generally catalyzes a chemical alteration of the chromogenic substrate which can be measured using various techniques. For example, the enzyme may catalyze a color change in a substrate, which can be measured spectrophotometrically. Alternatively, the enzyme may alter the fluorescence or chemiluminescence of the substrate. Techniques for quantifying a change in fluorescence are described above. The chemiluminescent substrate becomes electronically excited by a chemical reaction and may then emit light which can be measured (using a chemiluminometer, for example) or donates energy to a fluorescent acceptor. Examples of enzymatic labels include luciferases (*e.g.*, firefly luciferase and bacterial luciferase; U.S. Patent No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, malate dehydrogenase, urease, peroxidase such as horseradish peroxidase (HRPO), alkaline phosphatase, -galactosidase, glucoamylase, lysozyme, saccharide oxidases (*e.g.*, glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase), heterocyclic oxidases (such as uricase and xanthine oxidase), lactoperoxidase, microperoxidase, and the like. Techniques for conjugating enzymes to antibodies are described in O'Sullivan *et al.*, Methods for the Preparation of Enzyme-Antibody Conjugates for use in Enzyme Immunoassay, in *Methods in Enzym.* (ed J. Langone & H. Van Vunakis), Academic press, New York, 73:147-166 (1981).

Examples of enzyme-substrate combinations include, for example:

(i) Horseradish peroxidase (HRPO) with hydrogen peroxidase as a substrate, wherein the hydrogen peroxidase oxidizes a dye precursor (*e.g.*, orthophenylene diamine (OPD) or 3,3',5,5'-tetramethyl benzidine hydrochloride (TMB));

(ii) alkaline phosphatase (AP) with para-Nitrophenyl phosphate as chromogenic substrate; and

(iii) -D-galactosidase (-D-Gal) with a chromogenic substrate (*e.g.*, p-nitrophenyl- -D-galactosidase) or fluorogenic substrate 4-methylumbelliferyl- -D-galactosidase.

Numerous other enzyme-substrate combinations are available to those skilled in the art. For a general review of these, see U.S. Patent Nos. 4,275,149 and 4,318,980.

Sometimes, the label is indirectly conjugated with the antibody. The skilled artisan will be aware of various techniques for achieving this. For example, the antibody can be conjugated with biotin and any of the three broad categories of labels mentioned above can be conjugated with avidin, or *vice versa*. Biotin binds selectively to avidin and thus, the label can be conjugated with the antibody in this indirect manner. Alternatively, to achieve indirect conjugation of the label with the antibody, the antibody is conjugated with a small hapten (*e.g.*, digoxin) and one of the different types of labels mentioned above is conjugated with an anti-hapten antibody (*e.g.*, anti-digoxin antibody). Thus, indirect conjugation of the label with the antibody can be achieved.

In another embodiment of the invention, the anti-trkC antibody need not be labeled, and the presence thereof can be detected using a labeled antibody which binds to the anti-trkC antibody.

The antibodies of the present invention may be employed in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays. Zola, *Monoclonal Antibodies: A Manual of Techniques*, pp.147-158 (CRC Press, Inc. 1987).

Competitive binding assays rely on the ability of a labeled standard to compete with the test sample analyte for binding with a limited amount of antibody. The amount of trkC protein in the test sample is inversely proportional to the amount of standard that becomes bound to the antibodies. To facilitate determining the amount of standard that becomes bound, the antibodies generally are insolubilized before or after the competition, so that the standard and analyte that are bound to the antibodies may conveniently be separated from the standard and analyte which remain unbound.

Sandwich assays involve the use of two antibodies, each capable of binding to a different immunogenic portion, or epitope, of the protein to be detected. In a sandwich assay, the test sample analyte is bound by a first antibody which is immobilized on a solid support, and thereafter a second antibody binds to the analyte, thus forming an insoluble three-part complex. See, *e.g.*, U. S. Patent No. 4,376,110. The second antibody may itself be labeled with a detectable moiety (direct sandwich assays) or may be measured using an anti-immunoglobulin antibody that is labeled with a detectable moiety (indirect sandwich assay). For example, one type of sandwich assay is an ELISA assay, in which case the detectable moiety is an enzyme.

The antibodies may also be used for *in vivo* diagnostic assays. Generally, the antibody is labeled with a radionuclide (such as ^{111}In, ^{99}Tc, ^{14}C, ^{131}I, ^{125}I, ^{3}H, ^{32}P or ^{35}S) so that the cells or tissue of interest can be localized using immunoscintiography.

The antibodies may also be used as staining reagents in pathology, following techniques well known in the art.

The anti-trkC agonist antibodies of the present invention are believed to possess numerous advantages over NT-3 as therapeutic agents, including improved efficacy, improved pharmacokinetic properties (pK) and bioavailability, and lack of hyperalgesia following administration.

4. Pharmaceutical formulations

Therapeutic formulations of the antibodies of the present invention are prepared for storage by mixing the antibody having the desired degree of purity with optional physiologically acceptable carriers, excipients or stabilizers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.*, Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

The formulations herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

The active ingredients may also be entrapped in microcapsule prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsule and poly-(methylmethacylate) microcapsule, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.*, films, or microcapsule. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the Lupron Depot™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as

ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

An effective amount of an antibody of the present invention to be employed therapeutically will depend, for example, upon the therapeutic objectives, the route of administration, and the condition of the patient. Accordingly, it will be necessary for the therapist to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. A typical daily dosage might range from about 1 g/kg to up to 100 mg/kg or more, depending on the factors mentioned above. Typically, the clinician will administer a molecule of the present invention until a dosage is reached that provides the required biological effect. The progress of this therapy is easily monitored by conventional assays.

Administration may be by any conventional route known in the art including, without limitation, intravenous, subcutaneous, topical, intramuscular, intratracheal, intracerebral, intranasal, intrapulmonary, and intraparyngal administration.

4. Gene therapy

The nucleic acid encoding the antibodies of the present invention may also be used in gene therapy of various (chronic) neurodegenerative disorders and acute nerve cell injuries, especially genetically acquired peripheral neuropathies. Two basic approaches to gene therapy have evolved: *ex vivo* gene therapy and *in vivo* gene therapy. In *ex vivo* gene therapy, cells are removed from a subject and cultured *in vitro*. A functional replacement gene is introduced into the cells *in vitro*, the modified cells are expanded in culture, and then reimplanted in the subject. *In in vivo* gene therapy, the target cells are not removed from the subject. Rather, the transferred gene is introduced into cells of the recipient *in situ*, that is, within the recipient.

Several *ex vivo* gene therapy studies in humans have been reported and are reviewed, for example, in Anderson, Science 256:808-813 (1992), and Miller, Nature 357:455-460 (1992).

The viability of *in vivo* gene therapy has been demonstrated in several animal models, as reviewed in Felgner *et al.*, Nature 349:351-352 (1991). Direct gene transfer has been reported, for example, into muscle tissue (Ferry *et al.*, Proc Natl. Acad. Sci. 88:8377-8781 (1991); Quantin *et al.*, Proc. Natl. Acad. Sci. USA 89:2581-2584 (1992)); the arterial wall (Nabel *et al.*, Science 244:1342-1344 (1989)); and the nervous system (Price *et al.*, Proc. Natl. Acad. Sci. 84:156-160 (1987)).

Accordingly, the present invention also provides delivery vehicles suitable for delivery of a polynucleotide encoding an agonist anti-trkC antibody into cells (whether *in vivo* or *ex vivo*). Generally, a polynucleotide encoding an

antibody (e.g. linear antibody or antibody chains) will be operably linked to a promoter and a heterologous polynucleotide. Delivery vehicles suitable for incorporation of a polynucleotide encoding an antibody of the present invention for introduction into a host cell include non-viral vehicles and viral vectors. Verma and Somia, Nature 389:239-242 (1997).

A wide variety of non-viral vehicles for delivery of a polynucleotide encoding an antibody of the present invention are known in the art and are encompassed in the present invention. A polynucleotide encoding an anti-trkC antibody can be delivered to a cell as naked DNA (U.S. Patent No. 5,692,622; WO 97/40183). Alternatively, a polynucleotide encoding an anti-trkC antibody herein can be delivered to a cell associated in a variety of ways with a variety of substances (forms of delivery) including, but not limited to cationic lipids; biocompatible polymers, including natural polymers and synthetic polymers; lipoproteins; polypeptides; polysaccharides; lipopolysaccharides; artificial viral envelopes; metal particles; and bacteria. A delivery vehicle can be a microparticle. Mixtures or conjugates of these various substances can also be used as delivery vehicles. A polynucleotide encoding an antibody herein can be associated non-covalently or covalently with these various forms of delivery. Liposomes can be targeted to a particular cell type, e.g., to a glomerular epithelial cell.

Viral vectors include, but are not limited to, DNA viral vectors such as those based on adenoviruses, herpes simplex virus, poxviruses such as vaccinia virus, and parvoviruses, including adeno-associated virus; and RNA viral vectors, including, but not limited to, the retroviral vectors. Retroviral vectors include murine leukemia virus, and lentiviruses such as human immunodeficiency virus. Naldini *et al.*, Science 272:263-267 (1996).

Non-viral delivery vehicles comprising a polynucleotide encoding an anti-trkC antibody can be introduced into host cells and/or target cells by any method known in the art, such as transfection by the calcium phosphate coprecipitation technique; electroporation; electropermeabilization; liposome-mediated transfection; ballistic transfection; biolistic processes including microparticle bombardment, jet injection, and needle and syringe injection; or by microinjection. Numerous methods of transfection are known to the skilled worker in the field.

Viral delivery vehicles can be introduced into cells by infection. Alternatively, viral vehicles can be incorporated into any of the non-viral delivery vehicles described above for delivery into cells. For example, viral vectors can be mixed with cationic lipids (Hodgson and Solaiman, Nature Biotechnol. 14:339-342 [1996]); or lamellar liposomes (Wilson *et al.* Proc. Natl. Acad. Sci. USA 74:3471 [1977]; and Faller *et al.* J. Virol. 48:269 [1984]).

In a preferred embodiment, nucleic acid encoding both the heavy and the light chains (including fragments) of an anti-trkC antibody of the present invention will be present in the same polycistronic expression vector, such as those disclosed in U.S. Patent Nos. 4,965,196 and 4,713,339. Polycistronic expression vectors contain sequences coding for a secondary protein and a desired protein, wherein both the desired and secondary sequences are governed by the same promoter. The coding sequences are separated by translational stop and start signal codons. The expression of the secondary sequence effects control over the expression of the sequence for the desired protein, and the secondary protein functions as a marker for selection of transfected cells.

In *in vivo* gene therapy, the vector may be administered to the recipient, for example, by intravenous (i.v.) injection. Suitable titers will depend on a variety of factors, such as the particular vector chosen, the host, strength of promoter used, and the severity of the disease being treated.

The invention will be further illustrated by the following non-limiting examples.

EXAMPLES

Example 1 Production and characterization of agonist anti-trkC monoclonal antibodies

Production and Isotyping of Antibodies

Wild type Balb/C mice and transgenic mice producing human IgG2 or IgG4 (Xenomice, described in Mendez *et al., Nature Genetics* 15: 146-156 [1997]) were hyperimmunized either intraperitoneally, via rear footpad, or subcutaneously with 20 µg of human trkC-IgG (Shelton *et al., J. Neurosci.* 15: 477-491 (1995)) in either Freund's or Ribi adjuvant as described in Mendez et al. (*supra*). Spleen cells from the immune mice were fused with myeloma cells (X63.Ag8.653, ATCC Rockville, MD). A total of 33 fusions were performed using 253 Xenomice and 35 wild type Balb/C mice. Plates (21,734 wells total) were initially screened by direct ELISA using trkC-IgG. The ELISA screen identified 684 trkC positive hybridomas, all of which were then evaluated for agonist activity in trkC KIRA (Kinase activated Receptor Assay). The KIRA identified 14 Xenomouse derived and 22 wild type Balb/C mice derived hybridomas secreting anti-trkC agonist antibodies. These hybridomas were subcloned by limiting dilution, reassayed to confirm agonist activity, and were used to induce ascites by injecting into Pristane-primed Balb/C or nude mice (Hongo *et al., Hybridoma* 14: 253-260 [1995]). The monoclonal antibodies present in ascites were purified by Protein A affinity chromatography (Hongo *et al., supra*). Specific fusion efficiency (number of positivas / number of wells screened) was 3% for both the Xenomouse and wild type Balb/C mouse fusions. The incidence of agonist monoclonal antibodies (agonists / number of trkC ELISA positives) was 3% and 8% for the Xenomouse and wild type Balb/C mouse fusions, respectively. Isotypes of the murine monoclonal antibodies were determined using either GIBCO BRL dipstick or Zymed mouse-typer isotyping kit, following supplier's instructions. The Xenomice were either IgG_2 or IgG_4 strain, producing corresponding isotypes of antibodies. Table 1 shows isotypes of various human and murine anti-trkC monoclonal antibodies. A total of 8 human IgG_2, 6 human IgG_4, 7 murine IgG_1, 16 murine IgG_{2a} and 5 murine IgG_{2b} monoclonal antibodies were identified. The monoclonal antibodies with the most potent agonist activity (depicted by asterisk in Table 2), as determined by KIRA assay, were selected for in-depth characterization.

Table 2

Human Mabs (14 Total)

IgG_2 Isotype (8 Mabs)	IgG_4 Isotype (6 Mabs)
2.5.1*	4.8

	6.1.2*	2337
	8.4.1*	2338
	2342	2339
	2343	2348
5	2344 *	2349*
	2345 *	
	2346	

10 **Murine Mabs (22 Total)**

IgG_1 (7)	IgG_{2a} (10)	IgG_{2b} (5)	IgG_3
2249	2246*	2252	
2250*	2272	2273	
2253*	2251	2277	
2254	2265	2279	
2256*	2274	2280	
2257	2275		
2260	2276		
	2278		
	2281		
	2282		

Determination of Agonist Activity

a. *KIRA Assay*

Two bioassays were used to determine NT-3 agonist activity of anti-trkC monoclonal antibodies. The Kinase activated receptor assay (KIRA), which has been discussed in greater detail hereinabove, measures tyrosine phosphorylation of trkC in transfected cells in response to stimulation with a ligand, such as NT-3, or agonist monoclonal antibodies (Sadick *et al., Exp. Cell Res.* 234: 364-381 [1997]). The monoclonal antibodies were diluted to 27 µg/ml in KIRA stimulation buffer (F12/DMEM 50:50 containing 2% bovine serum albumin (BSA; Intergen Co., Purchase, NY) and 25 mM Hepes, 0.2 µm filtered). The monoclonal antibodies were further diluted serially 1:3 (8 dilutions total; concentrations ranging from 0.01-180 nM) in stimulation medium. Chinese Hamster Ovary (CHO) cells stably transfected with trkC fused with a 28 amino acid polypeptide flag epitope derived from HSV glycoprotein D (gD) were seeded (5 x 10^4 cells/well) and grown in 96-well cell culture plates. The cells were then stimulated with either NT-3 (as a positive control) or various anti-trkC monoclonal antibodies, using serial dilutions of 0.1; 1.56; 3.13; 6.25; 12.5; 25; 50 and 100 ng/ml. All dilutions were assayed in duplicate for 6 hours. The assay was carried out essentially as described in Sadick *et al.* (*supra*). Briefly, cells were lysed using Triton X-100 and trkC present in lysate captured in ELISA using antibodies against the gD epitope and phosphorylated trkC detected and quantitated using anti-phosphotyrosine antibodies suitably conjugated with enzyme. A monoclonal antibody not directed against trkC (anti-IL8 IgG_1 Xenomaus-derived human antibody or anti-gp120 IgG_1 murine monoclonal antibody) was used as a negative

control. As shown in Figure 1 (A and B), all the selected anti-trkC monoclonal antibodies could mimic the activity of NT-3 inasmuch as they could stimulate tyrosine phosphorylation of trkC receptor. The human anti-trkC monoclonal antibodies (Fig. 1A) showed more potent agonistic activity than the murine anti-trkC monoclonal antibodies (Fig. 1B). For example, the best human anti-trkC monoclonal antibody is 10-fold more potent than the best murine anti-trkC monoclonal antibody. Furthermore, the human monoclonals were nearly as efficient as NT-3 especially in the lower range of concentration.

b. *PC12 neurite outgrowth assay*

Another assay used to determine NT-3 mimetic activity of anti-trkC monoclonal antibodies was PC12 neurite outgrowth assay. This assay measures the outgrowth of neurite processes by rat pheocytochrome cells (PC12) in response to stimulation by appropriate ligands. These cells express endogenous trkA and are therefore responsive to NGF. However, they do not express endogenous trkC and are therefore transfected with trkC expression construct in order to elicit response to NT-3 and its agonists. PC12 cells were transfected (Urfer *et al., Biochem.* 36:4775-4781 [1997]; Tsoulfas *et al., Neuron* 10:975-990 [1993]) with full-length human trkC and plated in 96-well cell culture plates (1000 cells/well). Three days following transfection, anti-trkC monoclonal antibodies were added in triplicate (concentration ranging from 0.0002 to 2.7 nM) and incubated for an additional 3 days at 37°C. The cells were then analyzed by phase contrast microscopy and cells with neurites exceeding 2 times the diameter of the cell were counted. The human as well as the murine anti-trkC monoclonal antibodies could stimulate neurite outgrowth in PC12 cells as shown in Fig. 1 C and D. The human anti-trkC monoclonal antibodies (Fig. 1C) exhibited far more potent activity than the murine anti-trkC monoclonal antibodies (Fig. 1D) thus corroborating the results obtained in the KIRA assay. Furthermore, consistent with the KIRA assay results, the human anti-trkC monoclonal antibodies showed roughly similar stimulation as obtained with NT-3. The results obtained with the two bioassays described above demonstrate the ability of anti-trkC monoclonal antibodies to mimic the activity of NT-3, the natural ligand of trkC receptor.

Agonist activity of the monoclonal antibodies was ranked according to maximum induction of tyrosine phosphorylation and calculated EC50 of the phosphorylation curves in the KIRA assay and PC12 neurite outgrowth assay. Table 3 summarizes characteristics of various anti-trkC agonist monoclonal antibodies.

Table 3

MAb ID Human MAbs	Isotype	Agonist Activity KIRA/PC12	Binds Rat trkC	Immunoblot NR/Red. NR	Immunoblot NR/Red. Red.	Affinity Kd (nM)
2.5.1	G2	(+++/+++)	NO	++	++	12
6.1.2	G2	(++++/++++)	NO	+	+	12.5

6.4.1	G2	(++++/++++)	YES	+	+	12
2344	G2	(+++/+++)	NO	++	+	19
2345	G2	(++++/++++)	NO	++	++	12.1
2349	G4	(++++/++++)	NO	++	+	23
Murine MAbs						
2248	G2a	(+/+)	NO	+++	-	5.9
2250	G1	(++/++)	NO	++	++	8.7
2253	G1	(++/+)	NO	++	++	42
2256	G1	(+/+)	YES	++	+	62

Testing Specificity of anti-trkC antibodies

The specificity of anti-trkC monoclonal antibodies was tested using direct ELISA. The microtiter plates were coated overnight with immunoadhesin construct of the receptor trkA-IgG, trkB-IgG or trkC-IgG as capture antigens (described in Shelton *et al., J. Neurosci.* 15: 477-491 (1995)) using 100 µl of 1 µg/ml solution diluted in 50 mM carbonate buffer, pH 9.5. CD4-IgG (Capon *et al., Nature* 337: 525-531 (1989)) was used in place of capture antigen as a negative control. The coated plates were incubated for 1 hr at room temperature with various concentration of anti-trkC monoclonal antibodies (100 µl of 0.01 to 1 µg/ml) diluted in PBS containing 0.5% BSA and 0.05% Tween 20. After washing to remove excess unbound antibodies, appropriate HRP conjugate (human monoclonal antibodies: goat anti-human κ-HRP, 1:5000 diluted; murine monoclonal antibodies: goat anti-mouse IgG (Fc)-HRP, 1:5000 diluted) was added and incubated for 1 hr at room temperature. The plates were then washed, developed and read as previously described (Hongo *et al., Hybridoma* 14: 253-260 (1995)). Figure 2 shows a representative example using a human anti-trkC monoclonal antibody 6.1.2. The binding was highly specific to trkC, and no significant cross-reaction was observed with either trkA or trkB. Similarly, other human and mouse anti-trkC monoclonal antibodies showed specific recognition of trkC.

The binding of various anti-trkC agonist monoclonal antibodies to human trkC and rat trkC was compared using a direct ELISA essentially as described above except the capture antigen used for human trkC was trkC-gD instead of trkC-IgG. Results shown in Figure 3 indicate that among human anti-trkC monoclonal antibodies, only 6.4.1 significantly recognized rat trkC, rest were specific for human trkC. Similarly, among murine monoclonal antibodies, only 2256 recognized rat trkC to a significant extent while others showed specific recognition of human trkC only.

Affinity studies

Affinities of anti-trkC agonist monoclonal antibodies were determined using using ***BIAcore***-2000™ surface plasmon resonance (SPR) system (BIAcore, Inc., Piscataway, N.J.). CM5 biosensor chips were activated with N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier's instructions. In the first series of binding experiments, the antigen, gD-tagged trkC, was diluted into 10 mM sodium acetate buffer (pH 4.8), and injected over the activated chip at a concentration of 0.09 mg/mL. Using variable

exposure times, four ranges of antigen density were achieved: 14,000-17,000 response units (RU), 7000-9000 RU, 2000-3000 RU, and 400-600 RU. The chip was blocked with ethanolamine.

In the first series of kinetic measurements, anti-trkC antibodies (IgG's) were diluted into running buffer (PBS containing 0.05% Tween-20 and 0.01% sodium azide) and 0.03 mL (667 nM) was injected over the biosensor chip at 25°C at a flow rate of 0.01 mL/min. Regeneration was achieved with a 30 sec pulse of 10 mM HCl, followed by a 1 min pulse of 100 mM Tris-HCl, pH 8.0 and two wash steps.

In a second series of experiments, the IgG's (0.1 mg/mL in 10 mM sodium acetate, pH 4.8) were immobilized as described above, except that antibody density was limited to 1000-2000 RU. Two-fold serial dilutions of gD-trkC in the range of 3.7 M to 29 nM were then injected over the biosensor chip for kinetics measurements as described above.

The dissociation phase of each kinetic curve were fit to a single exponential dissociation rate (k_{off}), and these rates were used in the calculation of the association rate (k_{on}) from the injection phase, using a simple 1:1 Langmuir binding model (Lofas & Johnsson, 1990).

Equilibrium dissociation constants, K_d's, from SPR measurements were calculated as the ratio k_{off}/k_{on}.

The affinities of anti-trkC antibodies for gD-trkC were measured in SPR kinetics experiments with either antigen or antibody immobilized. Apparent affinities determined from experiments using low densities of immobilized antigen (0.4 to 0.6 ng/mm2), were generally consistent with those determined in experiments using immobilized IgG (see Table 2). However, at higher densities of immobilized gD-trkC, the apparent binding affinity of each antibody became progressively tighter by factors of as much as 10 fold, probably because of an avidity effect of binding by the bivalent IgG (data not shown). In some cases, no binding could be detected when trkC was injected over immobilized IgG. This may have occurred because immobilization of the IgG led to steric blocking of the antigen-binding site. Under all conditions tested, the antibody 2248 had the highest apparent affinity (K_d = 5.6 to 8.5 nM) of all antibodies tested.

Table 4

Binding affinities determined by SPR. Results are shown for IgG's binding to immobilized gD-trkC (400-600 RU) and for gD-trkC binding to immobilized IgG's (1000-3000 RU). NDB= no detectable binding.

	K_d (nM)	
Antibody (IgG)	Immobilized gD-trkC	Immobilized IgG
2248	5.9	8.5
2250	8.7	26
2253	42	51
2256	62	300
2344	19	NDB
2345	12	NDB
2349	23	NDB
6.4.1	12	28
6.1.2	13	16
2.5.1	12	NDB

Competition ELISA

A competition ELISA was used to get preliminary information about various groups to which these antibodies belong depending on the epitope(s) on trkC they recognize. In this assay, trkC-gD (1 μg/ml) was used as a capture antigen to coat microtiter plate. A specific biotinylated anti-trkC monoclonal antibody (1 μg/ml) was added to the coated plate either alone or in presence of another anti-trkC monoclonal antibody that was unlabeled and used in excess (50 μg/ml) as compared to the labeled antibody. If biotinylated antibody and unlabeled antibody both recognize the same or overlapping epitope, they will compete for binding to the immobilized trkC, resulting in decreased binding of the labeled antibody. If they recognize different and non-overlapping epitopes, there will be no competition between them, and the binding of the labeled antibody to the immobilized trkC will not be affected. Unlabeled human IgG2 and mouse IgG were used as negative control. A representative data in Figure 4 shows that all anti-trkC monoclonal antibodies, except murine anti-trkC 2248 monoclonal antibody, compete with labeled human anti-trkC 6.1.2 monoclonal antibody for binding to the immobilized trkC, suggesting that murine 2248 antibody recognizes an epitope on trkC that is different from the epitope(s) recognized by all other anti-trkC antibodies.

It is interesting to note that when unlabeled murine monoclonal antibody 2248 is bound first to immobilized trkC, none of the other (biotinylated) antibodies can access their binding site, suggeting that even though the epitopes are

distinct, steric hinderance may play a role. Such pairwise comparison gives valuable information and helps in classifying antibodies directed against the same antigen into different groups based on epitope recognition. A summary of such comparison is shown in Figure 5. The results indicate that the antibodies can be divided into two distinct groups: Group 1 encompasses all monoclonal antibodies except 2243, whereas Group 2 is composed of 2248.

Epitope Mapping with Domain Swap Mutants

Further epitope mapping was performed utilizing chimeric trkC in which various domains were replaced with corresponding domains from trkA or trkB. This approach was made possible by the fact that anti-trkC antibodies do not significantly cross-react with trkA or trkB. The use of such domain-swap mutants has a distinct advantage over deletion mutants. The deletion of a domain might disrupt the secondary structure of protein whereas substitution of a domain with a corresponding domain, of similar size and substantially similar amino acid sequence, from a related protein in domain-swap mutants is likely to retain the secondary structure. The extracellular domain of trk receptors is composed of 5 domains as shown in Figure 6A. D1 and D3 are cysteine-rich domains, D2 is a leucine-rich domain, and D4 and D5 are immunoglobulin-like domains. Domain-swap mutants of trkC containing replacement of D1, D4 and D5 with the corresponding domains from trkB or trkA were made (Urfer *et al., EMBO J.* 14:2795-2805 (1995)). Wild type trkC and wild type trkA were used as positive and negative controls respectively. The domain-swap mutants of trkC are designated according to the source of the replaced domain. For example, s1B has D1 domain from trkB, s4B has D4 domain from trkB, s5B has D5 domain from trkB, and s5A has D5 domain from trkA. All of the mutants were expressed as immunoadhesin, i.e. fused to IgG, and purified.

The binding of each of the agonist anti-trkC monoclonal antibody to various domain-swap mutants was evaluated by ELISA. $F(ab')_2$ fragment from goat anti-human IgG was used for coating microtiter plates to capture serial dilutions (100 μg/ml to 2.4 ng/ml, 100 μl/well, one hour at room temperature) of immunoadhesins (trkC-IgG, trkA-IgG and domain-swap mutants of trkC as immunoadhesin). Either unlabeled human or biotinylated murine anti-trkC monoclonal antibodies were added (100 μl per well; 1 μg/ml, one hour at room temperature) to the plates containing immobilized immunoadhesins, washed to remove unbound excess reagents, and incubated with goat anti-human κ or streptavidin conjugated to HRP. As shown in Figure 6B, all human anti-trkC monoclonal antibodies were able to recognize trkC domain-swap mutants with replacement of domain D1 or D4. However, replacement of domain D5 with the corresponding domain derived from either trkB or trkA destroyed recognition by anti-trkC antibodies. The extent of binding was reduced to the same low level as that observed with a negative control, trkA. The results suggest that all the human anti-trkC monoclonal antibodies tested recognize an epitope located somewhere in domain D5.

Similar analysis was performed with murine anti-trkC monoclonal antibodies essentially the same way except the secondary antibody used was goat anti-mouse IgG Fc coupled to HRP. As with the human anti-trkC antibodies, the replacement of domain D5 abolished binding to all the murine anti-trkC monoclonal antibodies tested (Figure 6B). Additionally, the replacement of domain D4 also destroyed the binding of 2248 murine anti-trkC antibody. The human

as well as murine anti-trkC agonist monoclonal antibodies all seem to recognize an epitope in domain 5 with the exception of 2248 murine antibody, which seems to additionally recognize a determinant in domain 4. It appears that 2248 epitope may be a linear epitope overlapping the boundary of domain 4 and 5. Alternatively, 2248 antibody might recognize a secondary structure formed by discontiguous epitope with determinants derived from both domain 4 and domain 5. Interestingly, Urfer *et al.* (J. Biol Chem. 273: 5829-5840 [1998]) have earlier established the prominent role of domain 5 in trkC receptor for mediating the interaction with NT-3. Surprisingly, the antibodies described herein also bind to an epitope of trkC which is largely overlapping with that recognized by NT-3. This is surprising because of the relative sizes and shapes of NT-3 and immunoglobulin molecules. The likely mode of action of these activators is to crosslink the extracellular domains of two trkC molecules in such a way to bring together their intracellular tyrosine kinase domains and cross phosphorylate and activate them.

In homodimeric NT-3, it has been established that the two areas of the molecule which interact with trkC are diametrically opposed on opposite sides of the molecule, 180 degrees apart from each other. The distance between these areas is on the order of 16 Å. On the other hand, the two trkC interacting sites in the immunoglobulin molecules described here are not diametrically opposed. In addition to displaying the trkC binding domains at a different angle than NT-3, immunoglobulins will have the trkC binding domains separated from each other by a much wider distance than they are in NT-3. This will vary with the exact angle of the two Fab domains, but is in the range of 50 Å to 150 Å. It would have been difficult to have foreseen that two such very different crosslinkers as NT-3 and the agonist Mabs act as agonists when bound to the same site on trkC.

Site-Directed Mutagenesis

Site-directed mutagenesis approach was used to determine the contribution of selected individual amino acid residues of domain 5 in the recognition by anti-trkC antibodies. Figure 7 shows the amino acid sequence of human trkC domains 4 and 5. All dotted residues were mutagenized to alanine except residues L284, L286 and E287 which were changed to E, H, and K respectively (Urfer *et al., J. Biol. Chem.* 273: 5829-5840 [1998]). A total of 26 single amino acid mutations were made and evaluated for their effect on binding to anti-trkC monoclonal antibodies. The values shown in Table 5 represent the ratio of binding to anti-trkC antibody of mutant *vs* wildtype trkC. In order to minimize variation and provide effective comparison, EC50 values were determined for each mutant for each antibody and divided by the EC50 value obtained with wildtype trkC.

Table 5

trkC Mutant	NT-3*	2.5.1	6.1.2	6.4.1	2344	2345	2349	2248	2250	2253	2256	1436
trkC	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
R275A	1.1											
E280A	0.7											
E283A	1.7											

L284E	1.1	NB	NB	NB	NB	NB	NB	0.7	0.6	0.7	1.1	0.8
R285A	1.5	1.1	1.2	0.8	0.9	1.0	0.9	0.9	2.6	0.9	NB	0.4
L286H	1.2	0.6	1.3?	NB	0.9	0.5	0.6	0.6	0.4	0.4	0.6	0.6
E287K	27.3	NB	NB	NB	NB	NB	NB	0.6	0.8	0.7	0.7	0.6
E291A	1.0											
R295A	11.6											
Q309A	1.0											
R312A	0.8											
K315A	0.9											
H318A	1.0	0.8	1.1	0.7	0.8	1.0	0.7	1.0	1.0	0.8	0.8	1
E320A	1.0											
E324A	1.2											
E329A	1.0											
N335A	37.8	NB	NB	0.3	NB	NB	1.5	0.6	0.5	0.5	0.5	0.6
K336A	0.9											
T338A	30.3											
H339A	1.7											
K350A	1.0											
Q358A	1.2											
K360A	0.9											
E367A	1.2											
D372A	1.2											
E373A	1.1											

The gray areas indicate that the designated mutants did not have an initial effect on monoclonal antibody binding, and were therefore not re-assayed. Mutations that completely obliterated monoclonal antibody binding are shown as NB ("no binding observed"). The analysis indicates the major contribution of amino acid residues L284, E287 and N335 of trkC in recognition by anti-trkC agonist monoclonal antibodies tested. A model of the complex of trkC domain 5 with NT3 shows the position of these residues in close contact with CDRs of antibody (Figure 8). This model is based on the crystal structure of the complex of trkA domain 5 with NGF. For further details see, e.g. Urfer *et al. J. Biol. Chem.* (1998), *supra*, or Ultsch *et al.*, J. Mol. Biol. 290:149-159 (1999).

Cloning and Sequencing of Antibody Variable Regions

In order to better understand the molecular basis of interaction between trkC and anti-trkC monoclonal antibodies, the heavy and light chain variable sequences of agonist antibodies were cloned and DNA sequence determined. Total RNA was isolated from hybridoma cells producing the human and murine anti-trkC antibodies using RNA isolation kit from Stratagene (La Jolla, CA). RNA was reverse transcribed into cDNA using SuperScript II system (Life Technologies, Inc., Gaithersburg, MD) and specific 3' primers based on framework 4 sequences derived from the respective heavy or light chain subgroup (Kabat and Wu, *J. Immunol.* 147: 1709-1719 [1991]). Subsequent PCR amplification was performed using AmpliTaq DNA polymerase (Perkin Elmer, Foster City, CA) in presence of 2.5 M DMSO with specific forward primers based on the N-terminal amino acid sequences of heavy and light chains and the same 3' primers used for cDNA synthesis. PCR products were subcloned into an $F(ab')_2$ vector containing both human

heavy and light chain constant regions (Carter *et al., Bio/Technology* 10: 163-167 (1992)). Five clones each of the V_H and V_L domains were sequenced and a consensus sequence was obtained.

Figure 9 shows the deduced amino acid sequences of heavy chain of anti-trkC agonist monoclonal antibodies (2250, SEQ ID NO: 42; 2253, SEQ ID NO: 43; 2256, SEQ ID NO: 44; 6.1.2, SEQ ID NO: 45; 6.4.1, SEQ ID NO: 46; 2345, SEQ ID NO: 47; and 2349, SEQ ID NO: 48). The deduced amino acid sequences of light chain of anti-trkC agonist monoclonal antibodies are shown in Figure 10 (2250, SEQ ID NO: 49; 2253, SEQ ID NO: 50; 2256, SEQ ID NO: 51; 6.1.2, SEQ ID NO: 53; 6.4.1, SEQ ID NO: 53; 2345, SEQ ID NO: 54; and 2349, SEQ ID NO: 55). In both Figure 9 and Figure 10 the Complementarity Determining Regions (CDRs) are labeled as CDR1, CDR2 and CDR3, and the corresponding amino acid residues are shown in bold face. Figure 11 summarizes the sequences of CDRs of heavy chain as well as light chain of various anti-trkC monoclonal antibodies along with designation of respective heavy and light chain variable family to which they belong.

Based on the determined amino acid sequences of the CDRs of the heavy and light chains of the anti-trkC agonist monoclonal antibodies, it is possible to provide a general formula for several of these regions. For the murine antibodies, the heavy chain CDR1 may be represented by the formula XaaWXaaXaaWVX (SEQ ID NO:37), wherein Xaa at position 1 is F or Y, Xaa at position 3 is I or M and Xaa at position 4 is E or H. The murine heavy chain CDR2 may be represented by the formula EIXaaPXaaXaaXaaXaaTNYNEKFKXaa (SEQ ID NO:38), wherein Xaa at position 3 is L or Y, Xaa at position 5 is G or S, Xaa at position 6 is S or N, Xaa at position 7 is D or G, Xaa at position 8 is N or R and Xaa at position 17 is G or S. The murine heavy chain CDR3 may be represented by the formula KNRNYYGNYVV (SEQ ID NO:12) or KYYYGNSYRSWYFDV (SEQ ID NO:13). For the human antibodies, the heavy chain CDR1 may be represented by the formula XaaXaaXaaYYWXaa (SEQ ID NO:39), wherein Xaa at position 1 is S or I, Xaa at position 2 is G or S and Xaa at position 3 is G, T or Y and Xaa at position 7 is S or N. The human heavy chain CDR2 may be represented by the formula XaaIXaaXaaSGSXaaTXaaNPSLKS (SEQ ID NO:40), wherein Xaa at position 1 is Y or R, Xaa at position 3 is Y or F, Xaa at position 4 is Y or T, Xaa at position 8 is S or R and Xaa at position 10 is N or Y. The human heavy chain CDR3 may be represented by DRDYDSTGDYYSYYGMDV (SEQ ID NO:14), DGGYSNPFD (SEQ ID NO:15) or the formula ERIAAAGXaaDYYYNGLXaaV (SEQ ID NO:41) wherein Xaa at position 8 is A or T and Xaa at position 16 is D or A.

The deduced amino acid sequence of heavy and light chain variable regions was confirmed by determination of N-terminal peptide sequence of these antibodies. Electroblotting onto Millipore Immobilon-PSQ membranes was carried out for 1 hr at 250 mA constant current in a BioRad Trans-Blot transfer cell (Matsudaira, *J. Biol. Chem.* 262: 10035-10038 (1987)). The PVDF membrane was stained with 0.1% Coomassie Blue R-250 in 50% methanol, 0.5 min. and destained for 2-3 min. with 10% acetic acid in 50% methanol. The mebrane was thoroughly washed with water and allowed to dry before storage at 20°C. Automated protein sequencing was performed on model 494A Perkin-Elmer sequencer (Perkin-Elmer Corporation, Foster City, CA) equipped with on-line PTH analyzer. Protein electroblotted onto PVDF membrane were sequenced in 6 mm micro glass cartridge. Peaks were integrated with Justice Innovation software using Nelson Analytical 760 interfaces. Sequence interpretation was performed on a DEC Alpha (Henzel *et*

al., J. Chromatography 404: 41-52 (1987)). Table 6 summarizes the classification of human and murine anti-trkC agonist monoclonal antibodies based on their N-terminal sequences.

Table 6

Human anti-trkC agonist mAbs	Heavy chain	Light chain
6.1.2	Subgroup II	Kappa I
6.4.1	Subgroup II	Kappa I
2345	Subgroup II	Kappa III
2349	Subgroup II	Kappa III
2.5.1	Subgroup II	Kappa I
2344	Subgroup II	Kappa I
Murine anti-trkC agonist mAbs		
2248	Subgroup IIA	Kappa I
2250	Subgroup IIA	Kappa I
2253	Subgroup IIA	Kappa IV
2256	Subgroup IIA	Kappa III

Example 2 Effect of agonist anti-trkC monoclonal antibodies on neuropathies in experimental animal model

The principal use of NT-3 agonists is in the treatment and/or prevention of peripheral neuropathies. It is known that large fiber myelinated sensory neurons, which are involved in mediating proprioception and vibration sense, express trkC that acts as a high affinity receptor for NT-3. Neuropathies involving these large fibers are common in diabetes and are also induced in response to certain chemotherapeutic agents particularly cisplatin and pyridoxine. NT-3 has shown efficacy in animal models of experimental diabetic neuropathy and cisplatin induced neuropathy. However, the use of NT-3 is severely hampered by its poor bioavailability as shown in a rodent model. The use of anti-trkC monoclonal antibodies as agonist of NT-3 offers numerous advantages and obviates a number of potential problems associated with the use of NT-3.

The *in vivo* half-life of agonist anti-trk monoclonal antibodies was determined by injecting either intravenously or subcutaneously in experimental animals. Shown on Figure 12 are serum levels of monoclonal antibody 2256 at various times after intravenous (IV) injection of 1 mg/kg or subcutaneous (subQ) injection of 5 mg/kg in rats. The serum levels were determined b using the KIRA assay to measure the amount of fully functional antibody 2256 by its ability to increase tyrosine autophosphorylation of trkC. These data indicate that monoclonal antibody 2256 in the rat has a half-life of 9 days and a bioavailability of 69% after subcutaneous administration. These values are consistent with those obtained with other antibodies, and are distinctly different from those obtained with NT3. Also shown in Figure 12 is data obtained after injection of NT-3 at the same doses and routes as shown for Mab 2256 (1

mg/kg, IV; 5 mg/kg subQ). These data indicate a serum half-life on the order of 4-5 minutes for NT-3, and a subcutaneous bioavailability of 7%. These data indicate that the antibodies are a significant improvement over NT-3 in terms of the very important properties of bioavailability and *in vivo* serum half-life.

It has been shown in two animal models of large fiber sensory neuropathy that NT-3 can protect or reverse the effects of chemical insult. Very high doses of NT3 have been shown to protect large fiber sensory neurons from the toxic effects of high doses of pyridoxine, and more moderate doses of NT3 have been shown to reverse the effects of cisplatinum administration. Since there might be many differences in the tissue distribution of NT-3 and the agonist Mabs described here, it is important to determine whether the *in vitro* activity of the Mabs translates into efficacy in animal models.

In order to create an animal model of cisplatinum induced neuropathy, adult rats were dosed with cisplatinum twice a week for sixteen weeks with 1 mg/kg intraperitoneally (IP). At this point, rats were split into four groups. All four groups continued receiving cisplatinum twice weekly. In addition to the continued cisplatinum, one group received NT-3 at a dose of 1mg/kg, three times per week, one group received Mab 2256 at a dose of 1 mg/kg once a week, one group received Mab 6.4.1 at a dose of 1 mg/kg once a week, and one group received saline three times a week. The NT-3 doses were given subcutaneously, while the Mabs and saline were administered IV. This treatment regime was continued for an additional four weeks, for a total of twenty weeks of cisplatinum administration.

The function of large fiber sensory neurons was assessed in these animals electrophysiologically, by use of H-wave recording (Gao *et al.*, Ann. Neurol. 38(1):30-7 [1995]. As can be seen from the data shown in Figure 13, the sensory conducton velocity was very low in the animals treated with cisplatinum with saline alone. NT-3 treatment three times a week caused an improvement of this lowered conduction velocity, as did treatment with either Mab 2256 or Mab 6.4.1 once a week. The magnitude of the improvement seen with the monoclonal antibodies used once a week was at least as great as that seen with three times a week treament with NT-3.

Pyridoxine is also known to induce a sensory neuropathy that primarily damages the large myelinated subpopulation of sensory neurons (Helgren *et al.*, J. Neurosci. 17(1):372-32 [1997]). High doses of NT3 have been shown to block the development of this neuropathy (Helgren *et al., supra*). Treatment of animals with two different doses of pyridoxine (either 400 mg/kg or 600 mg/kg daily, IP) for two weeks causes damage to the large neurons of the DRG. This damage can be detected by a decrease in the expression of several proteins known to be expressed either preferentially or exclusively by large neurons in the DRG. The expression level of these markers was assessed by measuring the level of the mRNA encoding them by use of the TAQMAN RT-PCR technique.

Taqmen RT-PCR for trkC agonist effects:

A. Probes and Primers

NFL

F-CAGCAGAACAAGGTCCTGGAA 21MER (SEQ ID NO: 72)
R-AGCGGGAAGGCTCTGAGTG 19MER (SEQ ID NO: 73)
P-AGCTGTTGGTGCTGGCCAGAA 22MER (SEQ ID NO: 74)

NSE
F-TCCATTGAAGACCCATTCGAC 21MER (SEQ ID NO: 75)
R-GCCGACATTGGCTGTGAAC 19MER (SEQ ID NO: 76)
P-AGGATGACTGGGCAGCTTGGTCCA 24MER (SEQ ID NO: 77)

TRKC
F-CAGCCCACTGCACCATATCA 20MER (SEQ ID NO: 78)
R-CTGTATCCGGCCCAGCAT 18MER (SEQ ID NO: 79)
P-CCATGGCATCACTACACCTTCATCGCT 27MER (SEQ ID NO: 80)

CALRET
F-TGGGAAAATTGAGATGGCAGA 21MER (SEQ ID NO: 81)
R-GCTGCCTGAAGCACAAAAGG 20MER (SEQ ID NO: 82)
P-CGCAGATCCTGCCAACCGAAGAGA 24MER (SEQ ID NO: 83)

PARVALB.
F-GACACCACTCTTCTGGAAAATGC 23MER (SEQ ID NO: 84)
R-TTGCCAAACCAACACCTACCA 21MER (SEQ ID NO: 85)
P-ATCGGACACCACCTGTAGGGAGGACC 26MER (SEQ ID NO: 86)

GAPDH
F-CAGTGGCAAAGTGGAGATTGT 21MER (SEQ ID NO: 87)
R-AATTTGCCGTGAGTGGAGTC 20MER (SEQ ID NO: 88)
P-CCATCAACGACCCCTTCATTGACCTC 26MER (SEQ ID NO: 89)

Probes and primers were designed using Primer Express, (ABI-Perkin-Elmer). Guidelines for primer probe selection are included in Williams and Tucker (1999) PCR applications, pp. 365-75 (Academic Press).

B. Total RNA Preparation and Quantification

L4 and L5 were dissected from phosphate buffered saline perfused rats. Left and right sides were isolated in separate tubes. For total RNA used in standard curves, all DRG were dissected from control rats. Total RNA was isolated using the Qiagen Rneasy mini columns. Tissue was homogenized as per manufacturers instructions. Total RNA was quantified utilizing the Ribogreen Quantitaion Kit (Molecular Probes) and following the manufacturers instructions.

C. RT-PCR

Twenty five nanograms of total RNA was used per 50ul reaction, except in standard curve reactions where 500, 250, 25 or 2.5 nanograms per reaction was used. Each reaction contained 25 pmol of each oligonucleotide primer, 0.2 mM of each dNTP, 100 nM flouresceatly labelled oligonucleotide probe, 1X RT-PCR buffer (PE biosystems), 2.0mM MgCl2, 20 U RNAse inhibitor, 12.5 MuLV reverse transcriptase (RT, PE biosystems) and 2.5U Amplitaq Gold polymerase (PE biosystems). Reverse transcription was performed for 30 min at 48 degrees C followed by 95 degrees C for 10 min for Amplitaq Gold activation and RT inactivation, then PCR; 40 cycles of 95 degrees C for 15 sec and 60 degrees C for one and a half minutes.

D. Gene Expression Quantitation

Control RNA was used to generate standard curves for a housekeeping gene and the genes of interest with each taqman run. A standard curve was obtained by plotting the threshold cycle (Ct) value obtained from the Taqman run versus the log of the quantity of control total RNA added. The resultant linear equation was solved for the log RNA value. Plugging in the experimental Ct value produced the log of the experimental gene expression value. Ten raised to the power of this value gives the experimental gene expression in nanograms.

As can be seen from Figure 14, pyridoxine treatment for two weeks resulted in a dose dependent decrease in neurofilament light chain (NFL), neuron specific enolase (NSE), trkC, and calretinin expression. Both the dose dependency and magnitude of these decreases varies from marker to marker, indicating a differential sensitivity of these proteins as markers of the neuronal damage.

In Figure 15 the results of treating animals with two doses of Mab 2256 along with the low dose (400 mg/kg daily) of pyridoxine are shown. NFL and NSE show a significant decrease in expression at this level of pyridoxine treatment. Cotreatment of animals with 5 mg/kg of Mab 2256 (subQ weekly) completely blocked this decrease in expression. A Mab 2256 dose of 1 mg/kg had no appreciable effect on the expression of these proteins. Neither trkC nor calretinin expression is significantly affected by this low dose pyridoxine treatment, but treatemtn with 5 mg/kg Mab 2256 actually increases trkC expression over control level.

When animals are treated with the higher pyridoxine dose of 800mg/kg daily, the expression of NFL, NSE and calretinin falls to very low levels, while trkC expression falls to about 50% of control values (Figure 16). Cotreatment with Mab 2256 at either 1 mg/kg or 5mg/kg significantly but not completely blocks the decrease in expression seen in trkC and calretinin. There is a slight trend towards protection seen with NFL and NSE expression in animals treated with Mab 2256, but it did not attain statistical significance. Thus, using multiple biochemical markers of damage to large sensory neurons, Mab 2256 is seen to be capable of ameliorating the toxicity of pyridoxine treatment.

In order to examine the electrophysiolgical and behavioral effects of pyridoxine neuropathy, rats were treated with twice daily injections of 400mg/kg pyridoxine for 8 days. The function of their large diameter sensory afferents were tested electrophysiologically by recording the M-wave (direct motor) and H-wave (reflex sensory) response in the muscles of the foot after stimulation of the sciatic nerve at the thigh and the calf (Gao *et al.*, Ann. Neurol. 38[1]:30-7 [1995]). Treatment with pyridoxine for 8 days resulted in a large decrease in the amplitude of the sensory response compared to the motor response as seen in Figure 18. Cotreatment with Mab 2256 significantly blocked the pyridoxine-induced decrease in the sensory amplitude. This is similar to effects published using very high doses (20mg/kg daily) of NT3 (Helgren *et al., supra*).

Animals treated with this regime of pyridoxine were also behaviorally tested for their proprioceptive function. They were trained to walk across a horizontal ladder in order to escape a bright light and white noise stimulus into a dark box. The animals were videotaped from below, and the quality of the placement of their hindpaws on the rungs of the ladder was read by an observer blind to their treatment. Each paw placement was scored as a good placement

(paw lands on forward part of metatarsals, immediately behind toes, with toes wrapping the rung immediately), solid landing (paw hits other than immediately behind toes, but solidly on rung, toes often not wrapping), near footfault (paw barely hits rung, either on the extreme forward part of toes or rearward aspect of heel, but does support weight) or footfault (paw either misses rung entirely or poor enough placement that foot does not support weight and falls through ladder upon weight bearing). Normal rats very quickly learn to place their hindpaws correctly, which requires an excellent proprioceptive sense of where the hindpaw is in space. After treatment with pyridoxine (400mg/kg twice daily for 8 days), the performance on this task had declined, with an almost thirty percent decline in good placements and an increase in both footfaults and near footfaults (Figure 18). Cotreatment of the animals with Mab 2256 during this time, allowed the animals to maintain a much higher degree of performance, with a smaller decline in good placements and smaller increases in footfaults and near footfaults.

In summary, cotreatment with Mab 2256 ameliorates the toxic effects of pyridoxine as measured biochemically, electrophysiologically, and by performance on a behavioral task.

After establishing that the trkC Mabs were therapeutically at least as effective as NT-3, the observed adverse event of hyperalgesia was examined. This side effect of NT-3 administration has been seen in rodents (see Figure 19) and in humans (Chaudhary *et al.*, Muscle and Nerve 23:189-192 (2000)). Rats were trained and tested for thermal sensitivity of the hind paws using a Hargreaves device and then administered 1mg/kg of Mab 2256 IV, or 1mg/kg NT-3 subcutaneously in the scruff. At two, four, and six hours after administration, the rats were again tested for their thermal withdrawal times. As can be seen from Figure 19, NT-3 administration caused a significant heat hyperalgesia at four and six hours post dosing, while the trkC Mab 2256 was without any effect on thermal pain sensation. So, at doses known to be effective in reversing or preventing neuropathyy, NT-3 does cause an increase in sensitivity to pain, while the Mab 2256 does not.

Cisplatin, a widely used chemotherapeutic agent, induces a sensory neuropathy with selective loss of vibration sense and proprioception. Here we demonstrate that neurotrophin-3 (NT-3), a member of the nerve growth factor family of neurotrophic factors, restored to normal levels the reduced H-reflex-related sensory nerve conduction velocity induced by cisplatin in rats. NT-3 treatment corrected an abnormal cytoplasmic distribution of neurofilament protein in large sensory neurons in dorsal root ganglia and the reduction in the numbers of myelinated fibers in sural nerves caused by cisplatin. The NT-3-dependent reversal of cisplatin neurotoxicity thus suggests the possible use of NT-3 in the treatment of peripheral sensory neuropathy.

Chronic treatment of adult rats for 2-3 weeks with high doses of pyridoxine (Vitamin B6) produced a profound proprioceptive loss, similar to that found in humans overdosed with this vitamin or treated with the chemotherapeutic agent cisplatin. Pyridoxine toxicity was manifest as deficits in simple and precise locomotion and sensory nerve function and as degeneration of large-diameter/large-fiber spinal sensory neurons. As assessed quantitatively in a beam-walking task and by EMG recording of H waves evoked by peripheral nerve stimulation, coadministration of the neurotrophic factor neurotrophin-3 (NT-3; 5-20 mg/kg/day, s.c.) during chronic pyridoxine treatment largely attenuated the behavioral and electrophysiological sequalae associated with pyridoxine toxicity.

Furthermore, NT-3 administration prevented degeneration of sensory fibers in the dorsal columns of the spinal cord. These data are consistent with the evidence that NT-3 is a target-derived neurotrophic factor for muscle sensory afferents and suggest that pharmacological doses of NT-3 may be beneficial in the treatment of large-fiber sensory neuropathies.

Deposit of Biological Material

The following hybridoma cell lines and plasmids have been deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209, USA (ATCC) on June 21, 2000:

Hybridoma/Plasmid Designation	ATCC No.
2.5.1	PTA-2151
6.1.2	PTA-2143
6.4.1	PTA-2150
2344	PTA-2144
2345	PTA-2148
2349	PTA-2153
2248	PTA-2147
2250	PTA-2149
2253	PTA-2145
2256	PTA-2152
DNA pXCA-2250HL	PTA-2136
DNA pXCA-2253HL	PTA-2137
DNA pXCA-2256HL	PTA-2138
DNA pXCA-6.1.2H	PTA-2141
DNA pXCA-6.4.1H	PTA-2143
DNA pXCA-2345H	PTA-2142
DNA pXCA-2349H	PTA-2133
DNA vegf4chim-6.1.2L	PTA-2134
DNA vegf4chim-6.4.1L	PTA-2135
DNA vegf4chim-2345L	PTA-2139
DNA vegf4chim-2349L	PTA-2140

This deposit was made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of viable cultures for 30 years from the date of the deposit. The organisms will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Genentech, Inc. and

ATCC, which assures permanent and unrestricted availability of the progeny of the cultures to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 USC §122 and the Commissioner's rules pursuant thereto (including 37 CFR §1.12 with particular reference to 886 OG 638).

In respect of these designations in which a European patent is sought, a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample. (Rule 28(4) EPC)

The assignee of the present application has agreed that if the cultures on deposit should die or be lost or destroyed when cultivated under suitable conditions, they will be promptly replaced on notification with a viable specimen of the same culture. Availability of the deposited strain is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by the constructs deposited, since the deposited embodiments are intended to illustrate only certain aspects of the invention and any constructs that are functionally equivalent are within the scope of this invention. The deposit of material herein does not constitute an admission that the written description herein contained is inadequate to enable the practice of any aspect of the invention, including the best mode thereof, nor is it to be construed as limiting the scope of the claims to the specific illustrations that they represent. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

It is understood that the application of the teachings of the present invention to a specific problem or situation will be within the capabilities of one having ordinary skill in the art in light of the teachings contained herein. Examples of the products of the present invention and representative processes for their isolation, use, and manufacture appear below, but should not be construed to limit the invention.

All references cited throughout the specification and the references cited therein are hereby expressly incorporated by reference.

WHAT IS CLAIMED IS:

1. An agonist anti-trkC monoclonal antibody which

(a) shows no significant cross-reactivity with trkA or trkB; and

(b) recognizes an epitope in domain 5 of trkC.

2. The antibody of claim 1 which further recognizes an epitope in domain 4 of trkC.

3. The antibody of claim 1 which binds both human and rat trkC.

4. The antibody of claim 1 which is a human antibody.

5. The antibody of claim 1 which is a murine antibody.

6. The antibody of claim 5 which is humanized.

7. The antibody of claim 1 effective in the treatment of cisplatin- or pyridoxine-induced neuropathy.

8. The antibody of claim 1 effective in the treatment of diabetic neuropathy.

9. The antibody of claim 1 which does not cause hyperalgesia when administered to a patient.

10. The antibody of claim 1 which has increased bioavailability as compared to NT-3.

11. The antibody of claim 1 which has a higher specific activity than NT-3.

12. An anti-trkC antibody heavy chain comprising the following CDR's: a CDR1 selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4 and 5; a CDR2 selected from the group consisting of SEQ ID NOs: 6, 7, 8, 9, 10 and 11; and a CDR3 selected from the group consisting of SEQ ID NOs: 12, 13, 14, 15, 16 and 17.

13. An anti-trkC antibody light chain comprising the following CDR's: a CDR1 selected from the group consisting of SEQ ID NOs: 18, 19, 20, 21, 22, 23 and 24; a CDR2 selected from the group consisting of SEQ ID NOs: 25, 26, 27, 28, 29 and 30; and a CDR3 selected from the group consisting of SEQ ID NOs: 31, 32, 33, 34, 35 and 36.

14. A murine anti-trkC antibody heavy chain comprising the following CDR's:

(a) a CDR1 of the formula XaaWXaaXaaWVK (SEQ ID NO: 37), wherein Xaa at position 1 is F or Y; Xaa at position 3 is I or M; and Xaa at position 4 is E or H;

(b) a CDR2 of the formula EIXaaPXaaXaaXaaXaaTNYNEKFKXaa (SEQ ID NO: 38), wherein Xaa at position 3 is L or Y; Xaa at position 5is G or S; xaa at position 6 is S or N; Xaa at position 7 is D or G; Xaa at position 8 is N or R and Xaa at position 17 is G or S; and

(c) a CDR3 of the formula KMRNYYGNYYV (SEQ ID NO: 12) or KYYYGNSYRSWYFDV (SEQ ID NO: 13).

15. A human anti-trkC antibody heavy chain comprising the following CDR's:

(a) a CDR1 of the formula XaaXaaXaaYYWXaa (SEQ ID NO: 39), wherein Xaa at position 1 is S or I; Xaa at position 2 is G or S; Xaa at position 3 is G, T or Y, and Xaa at position 7 is S or N;

(b) a CDR2 of the formula XaaIXaaXaaSGSXaaTXaaNPSLKS (SEQ ID NO: 40), wherein Xaa at position 1 is Y or R; Xaa at position 3 is Y or F; Xaa at position 4 is Y or T; Xaa at position 8 is S or R; and Xaa at position 10 is N or Y; and

(c) a CDR3 of the formula selected from the group consisting of DRDYDSTGDYYSYYGMDV (SEQ ID NO: 14); DGGYSNPFD (SEQ ID NO: 15); ERIAAADXaaDYYYNGLXaaV (SEQ ID NO: 41), wherein Xaa at position 8 is A or T and Xaa at position 16 is D or A.

16. An anti-trkC agonist monoclonal antibody comprising a heavy chain comprising the CDR's of the murine anti-trkC antibody heavy chain of claim 14 associated with a light chain.

17. The antibody of claim 16 comprising human framework residues.

18. The antibody of claim 17 which shows no significant cross-reactivity with trkA or trkB.

19. The antibody of claim 17 having a homo-tetrameric structure composed of two disulfide-bonded antibody heavy chain-light chain pairs.

20. The antibody of claim 17 which is an antibody fragment selected from the group consisting of Fv, Fab, Fab' and $F(ab')_2$ fragments.

21. An anti-trkC agonist monoclonal antibody comprising a human anti-trkC antibody heavy chain of claim 15 associated with a human antibody light chain.

22. The antibody of claim 21 which shows no significant cross-reactivity with trkA or trkB.

23. The antibody of claim 22 having a homo-tetrameric structure composed of two disulfide-bonded antibody heavy chain-light chain pairs.

24. The antibody of claim 22 which is an antibody fragment selected from the group consisting of Fv, Fab, Fab' and $F(ab')_2$ fragments.

25. The antibody of claim 22 which is an IgG.

26. The antibody of claim 25 which is an IgG-2 or IgG-4.

27. A murine anti-trkC agonist antibody selected from the group consisting of antibodies 2248, 2250, 2253 and 2256.

28. A human anti-trkC agonist antibody selected from the group consisting of antibodies 6.1.2, 6.4.1, 2345, 2349, 2.5.1 and 2344.

29. The antibody of claim 28 selected from the group consisting of antibodies 6.1.2, 6.4.1, 2345 and 2349.

30. An isolated nucleic acid molecule encoding a murine anti-trkC agonist antibody of claim 27.

31. An isolated nucleic acid molecule encoding a human anti-trkC agonist antibody of claim 28.

32. A nucleic acid molecule deposited with ATCC on June 21, 2000 under an accession number selected from the group consisting of PTA-2136, PTA-2137 and PTA-2138.

33. A nucleic acid molecule deposited with ATCC on June 21, 2000 under an accession number selected from the group consisting of PTA-2133, PTA-2134, PTA-2135, PTA-2139, PTA-2140, PTA-2141, PTA-2142 and PTA-2143.

34. A vector comprising a nucleic acid molecule of any one of claims 30-33.

35. A host cell line transformed with a nucleic acid molecule of any one of claims 30-33.

36. A hybridoma cell line transformed with a nucleic acid molecule of any one of claims 30-33.

37. An antibody produced by a hybridoma cell line of claim 36.

38. An isolated nucleic acid molecule encoding a heavy chain of an anti-trkC agonist monoclonal antibody, said nucleic acid molecule selected from the group consisting of SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO. 68 and SEQ ID NO: 70.

39. An isolated nucleic acid molecule encoding a light chain of an anti-trkC agonist monoclonal antibody, said nucleic acid molecule selected from the group consisting of SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69 and SEQ ID NO: 71.

40. A vector comprising a nucleic acid molecule of claim 38 or claim 39.

41. A host cell transformed with a nucleic acid molecule of claim 38 or claim 39.

42. A host cell transformed with both a nucleic acid molecule of claim 38 and a nucleic acid molecule of claim 39.

43. A hybridoma cell line transformed with a nucleic acid molecule of claim 38 or claim 39.

44. A hybridoma cell line transformed with a nucleic acid molecule of claim 38 and a nucleic acid molecule of claim 39.

45. An antibody produced by a hybridoma cell line of claim 43.

46. An antibody produced by a hybridoma cell line of claim 44.

47. A polypeptide encoded by a nucleic acid molecule of claim 38 or claim 39.

48. A polypeptide comprising polypeptide chains encoded by a nucleic acid molecule of claim 38 and a nucleic acid molecule of claim 39.

49. A pharmaceutical composition comprising an effective amount of an agonist anti-trkC monoclonal antibody of any one of claims 1, 16, and 21, in admixture with a pharmaceutically acceptable carrier.

50. A method for treating a neuropathy or neurodegenerative disease, or repairing a damaged nerve cell comprising administering to a mammal an effective amount of an antibody of claim 1.

51. The method of claim 50 wherein said neuropathy is selected from the group consisting of a peripheral neuropathy, diabetic neuropathy, and a large-fiber sensory neuropathy.

52. The method of claim 50 wherein said neurodegenerative disease is amyotrophic lateral sclerosis (ALS).

53. The method of claim 50 wherein said nerve cell is a sensory neuron or motor neuron.

54. The method of claim 53 wherein said sensory neuron is from dorsal root ganglia.

55. The method of claim 53 wherein said motor neuron is from the spinal cord.

56. The method of claim 50 wherein said administration is intravenous or subcutaneous.

57. The method of claim 50 wherein said administration is topical.

58. A method for enhancing the proliferation, maintenance or regeneration of peripheral neurons comprising contacting said neurons with an effective amount of an antibody of claim 1, claim 16, or claim 21.

59. A method for the treatment of a disease or condition involving cell degeneration in a mammalian subject by introducing nucleic acid encoding an anti-trkC antibody of claim 1 into a cell of such subject.

60. The method of claim 59 wherein the disease or condition selected from the group consisting of a neuropathy, a neurodegenerative disease, and nerve cell damage.

61. The method of claim 59 wherein said cell is a nerve cell.

62. The method of claim 59 wherein said nucleic acid is introduced into said cell *ex vivo*.

63. The method of claim 59 wherein said nucleic acid is introduced into said cell *in vivo*.

64. A method of inducing angiogenesis comprising delivering an anti-trkC antibody of claim 1 in an amount effective to induce angiogenesis.

65. A method of making an agonist anti-trkC antibody comprising preparing an antibody specifically binding trkC at a site overlapping with the binding site of a native NT-3 ligand of said trkC.

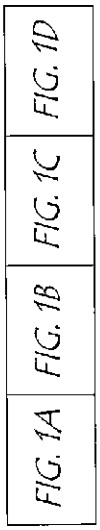
66. The method of claim 65 wherein said antibody binds trkC at essentially the same site as a native NT-3 ligand of said trkC.

67. The method of claim 65 wherein said antibody binds to an epitope within domain 5 of trk C.

68. The method of claim 67 wherein said trkC is a native human trkC polypeptide.

69. The method of claim 68 wherein said epitope includes amino acid residues L284, E287, and N335 of said native human trkC.

FIG. 1



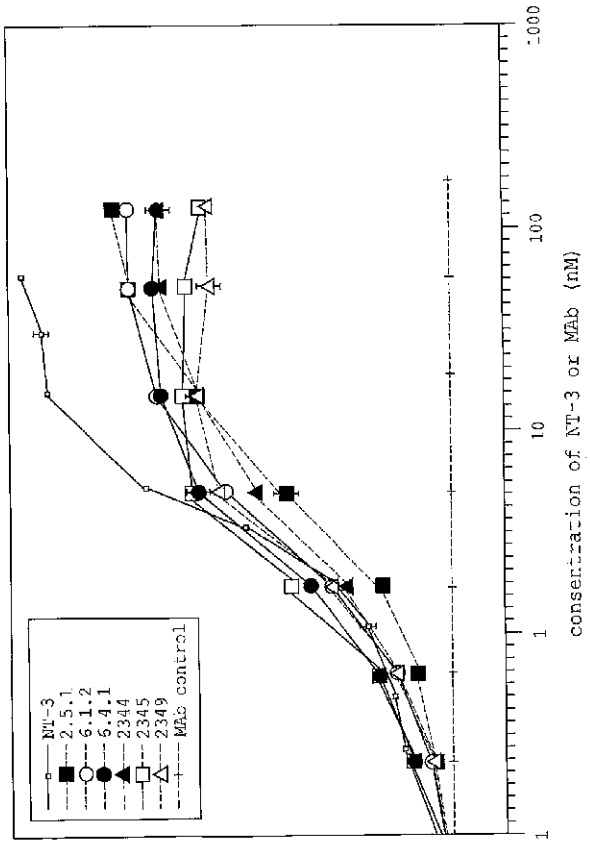

NT-3
2.5.1
6.1.2
6.4.1
2344
2345
2349
MAb control
1
10
100
1000
concentration of NT-3 or MAb (nM)

FIG. 1A

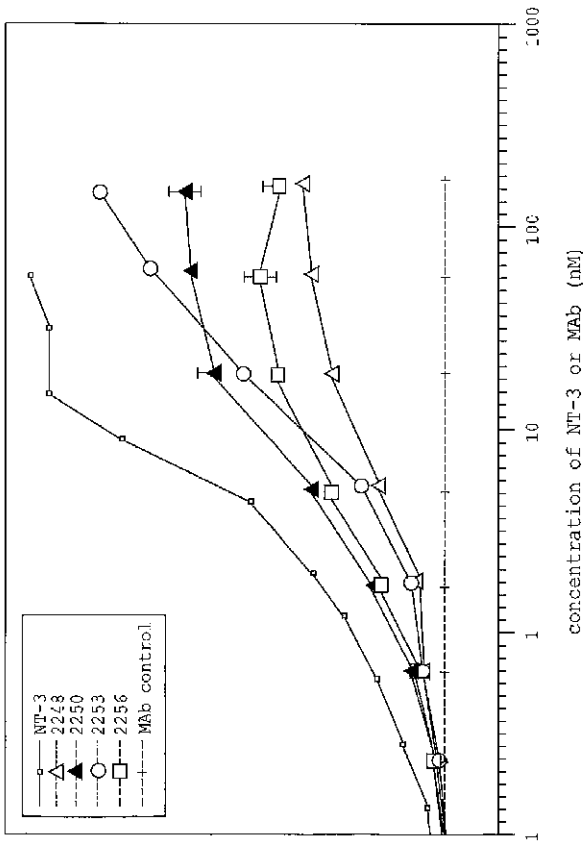

NT-3
2248
2250
2253
2256
MAb control
1
10
100
1000
concentration of NT-3 or MAb (nM)

FIG. 1B

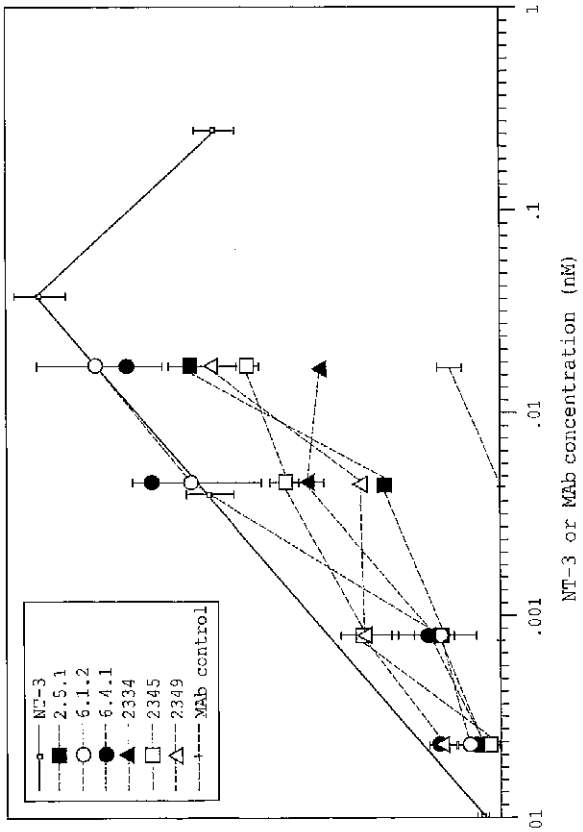

NT-3
2.5.1
6.1.2
6.4.1
2334
2345
2349
MAb control
1
.1
.01
.001
01
NT-3 or MAb concentration (nM)

FIG. 1C

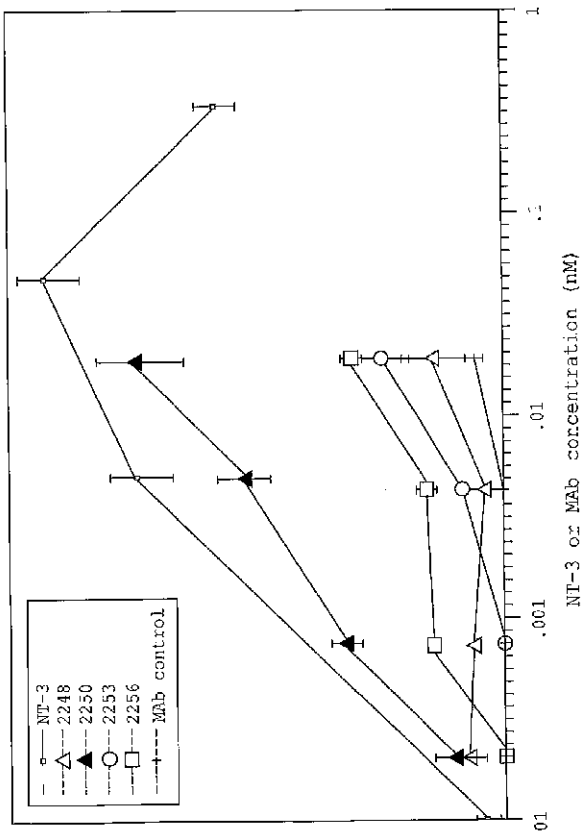

NT-3
2248
2250
2253
2256
MAb control
NT-3 or MAb concentration (nM)
1
.1
.01
.001
01

FIG. 1D

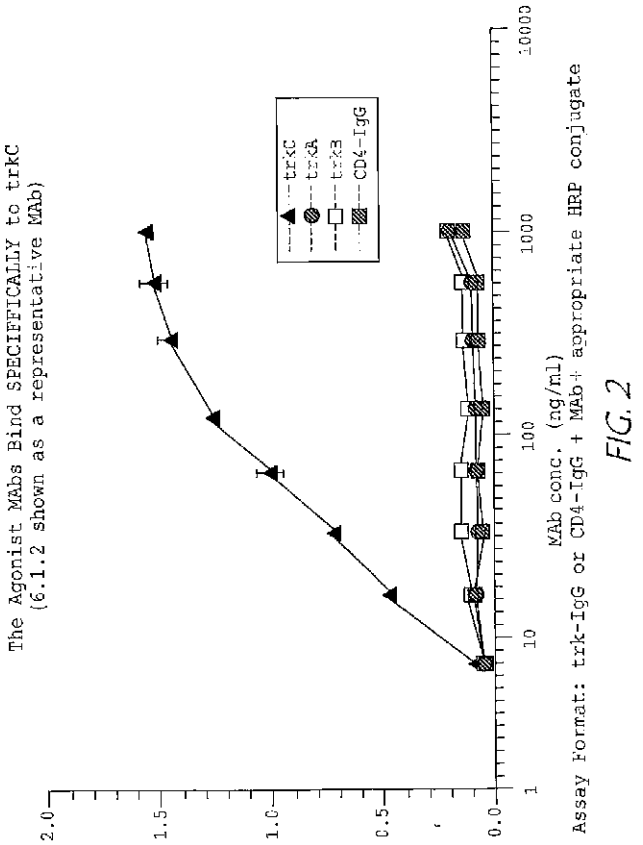

The Agonist MAbs Bind SPECIFFICALLY to trkC
(6.1.2 shown as a representative MAb)
trkC
trkA
trkB
CD4-IgG
2.0
1.5
1.0
0.5
0.0
1
10
100
1000
10000
MAb conc. (ng/ml)
Assay Format: trk-IgG or CD4-IgG + MAb+ appropriate HRP conjugate

FIG. 2

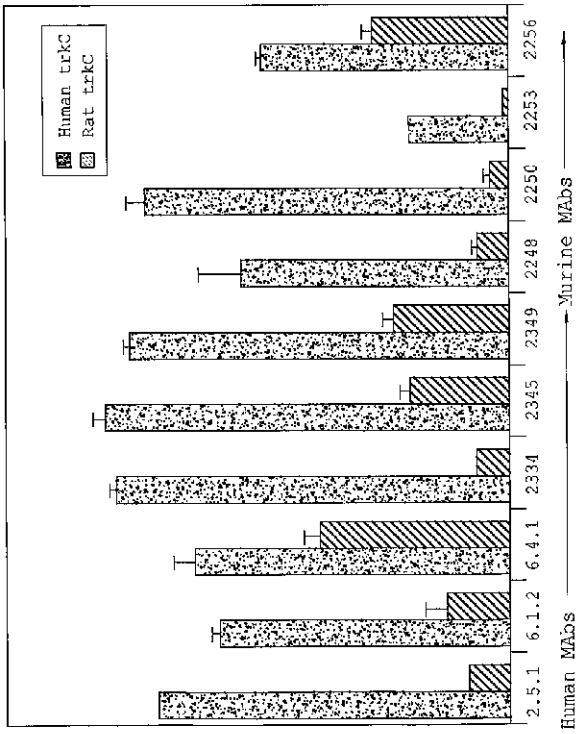

Human trkC
Rat trkC
2.5.1
6.1.2
6.4.1
2334
2345
2349
2248
2250
2253
2256
Human MAbs
Murine MAbs

FIG. 3

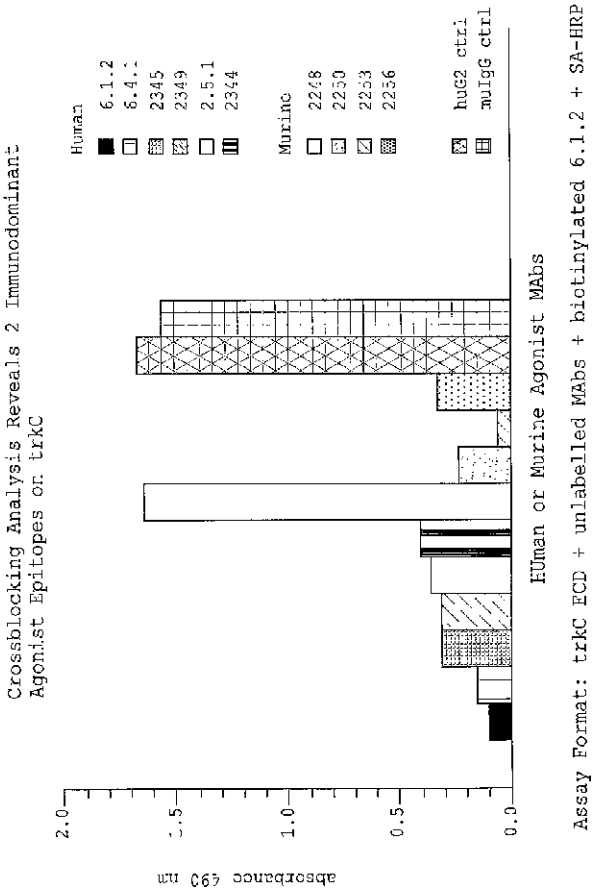
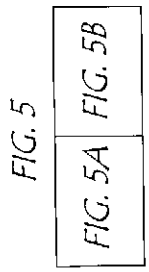

Crossblocking Analysis Reveals 2 Immunodominant Agonist Epitopes on trkC
Human
6.1.2
6.4.1
2345
2349
2.5.1
2344
Murine
2248
2250
2253
2256
huG2 ctrl
muIgG ctrl
absorbance 490 nm
2.0
1.5
1.0
0.5
0.0
HUman or Murine Agonist MAbs
Assay Format: trkC ECD + unlabelled MAbs + biotinylated 6.1.2 + SA-HRP

FIG. 4


FIG. 5
FIG. 5A
FIG. 5B

Epitope Mapping Summary

		unlabelled MAb									
	2344	2345	2349	2.5.1	6.1.2	6.4.1	2248	2250	2253	2256
biotin-MAb										
2344	0.02	0	0	0.01	0	0	1.52	0	0	0
2345	0.26	0.04	3.02	0.17	0.11	0.08	1.48	0.04	0.07	0.05
2349	0.09	0.01	0	0.06	0.04	0.03	1.55	0.02	0.03	0.02
2.5.1	0.06	0.01	0.01	0.03	0.03	0.02	1.54	0.01	0.02	0.01
6.1.2	0.3	0.24	0.24	0.27	0.08	0.12	1.55	0.2	0.05	0.23
6.4.1	0.2	0.12	0.1	0.15	0.06	0.07	1.56	0.1	0.03	0.1
2248	1.52	1.53	1.54	1.5	0.74	1.12	0.01	1.4	0.04	1.48
2250	0.27	0.1	0.05	0.21	0.08	0.07	1.57	0.01	0.07	0.21
2253	1.35	1.4	1.45	1.4	0.66	0.9	1.55	1.24	0	1.34
2256	0.52	0.15	0.07	0.38	0.13	0.12	1.63	0.01	0.02	0.02

FIG. 5A

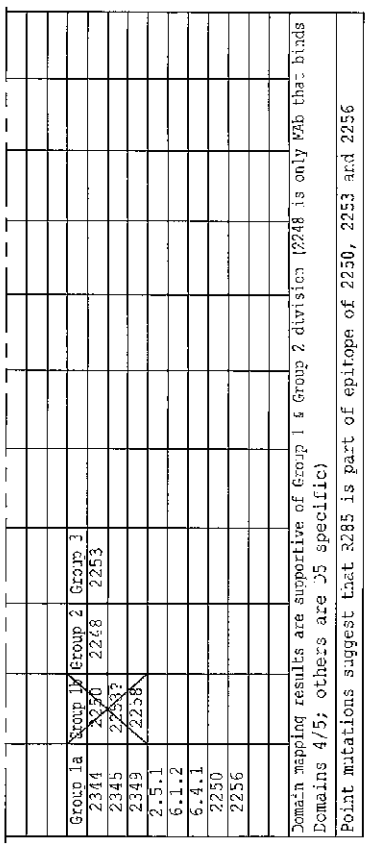

Group 1a
2344
2345
2349
2.5.1
6.1.2
6.4.1
2250
2256
Group 1b
2250
2253?
2258
Group 2
2248
Group 3
2253
Domain mapping results are supportive of Group 1 & Group 2 division (2248 is only MAb that binds Domains 4/5; others are J5 specific)
Point mutations suggest that R285 is part of epitope of 2250, 2253 and 2256
Assay Format: gd-crkC + unlabelled MAb + biotinylated MAb - SA-HRP
(1mg/ml) (50mg/ml) (1mg/ml) (1:1K)

FIG. 5B

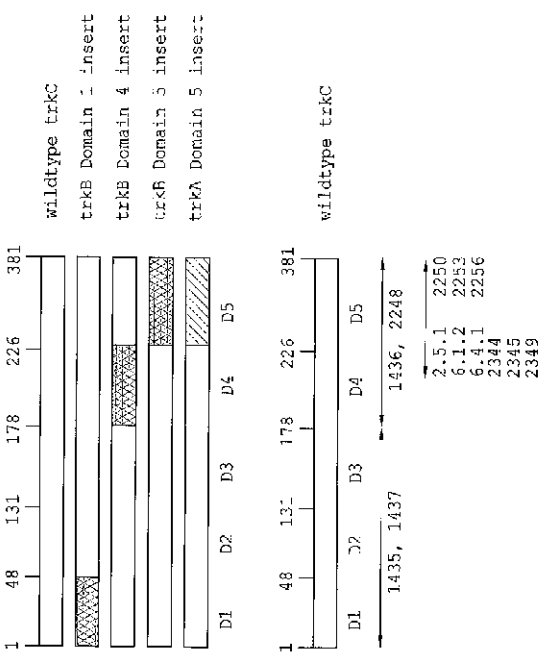

wildtype trkC
trkB Domain 3 insert
trkB Domain 4 insert
trkB Domain 5 insert
trkA Domain 5 insert
1
48
131
178
226
381
D1
D2
D3
D4
D5
1435, 1437
1436, 2248
2.5.1
6.1.2
6.4.1
2344
2345
2349
2250
2253
2256

FIG. 6A

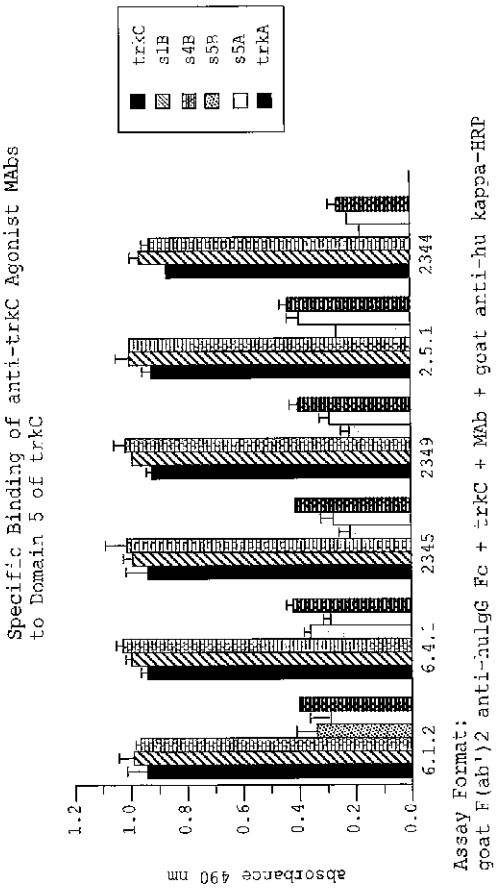

Specific Binding of anti-trkC Agonist MAbs
to Domain 5 of trkC
trkC
s1B
s4B
s5B
s5A
trkA
absorbance 490 nm
1.2
1.0
0.8
0.6
0.4
0.2
0.0
6.1.2
6.4.2
2345
2349
2.5.1
2344
Assay Format:
goat F(ab')2 anti-hulgG Fc + trkC + Mab + goat anti-hu kappa-HRP

FIG. 6B

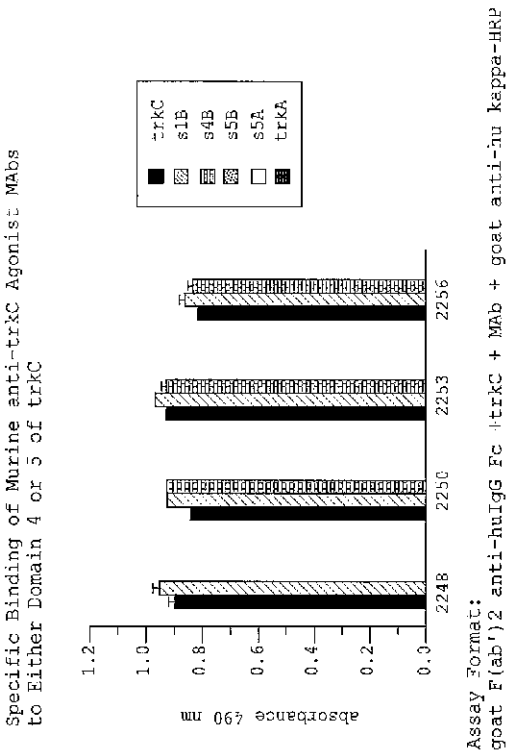

Specific Binding of Murine anti-trkC Agonist MAbs
to Either Domain 4 or 5 of trkC
trkC
s1B
s4B
s5B
s5A
trkA
absorbance 490 nm
1.2
1.0
0.8
0.6
0.4
0.2
0.0
2248
2250
2253
2256
Assay Format:
goat F(ab')2 anti-huIgG Fc +trkC + MAb + goat anti-lu kappa-HRP

FIG. 6C

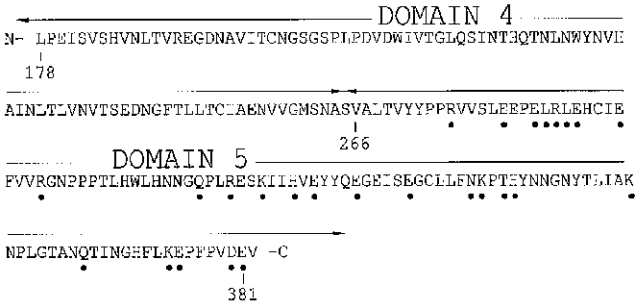

DOMAIN 4
N- LPEISVSHVNLTVREGDNAVITCNGSGSPLPDVDWIVTGLQSINTHQTNLNWYNVI
178
AINLTLVNVTSEDNGFTLLTCLAENVVGMSNASVALTVYYPPRVVSLEEPELRLEHCIE
266
DOMAIN 5
FVVRGNPPPTLHWLHNNGQPLRESKIIHVEYYQEGEISEGCLLFNKPTEYNNGNYTLIAK
NPLGTANQTINGHFLKEPFPVDEV -C
381

FIG. 7



FIG. 8

Heavy chain seqeces of anti-rkC agonist MAbs

CDR1 CDR2

2250 : N-QVQLQQSGAELMQPGASVKISCKSTGYTFSNFWIEWVKQRPG--HGLEWIGEILPGSDNTNYNEKFKG
2253 : N-QVQLQQSGAELMQPGASVKISCKSTGYTFSNFWIEWVKQRPG--HGLEWIGEILPGSDNTNYNEKFKG
2256 : N-QVQLQQPGAELVKPGASVKISCKATGYTFTSYWMHWVKQRPG--QGLEWIGEIYPSNGRTNYNEKFKS

6.1.2 : N-QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGGYYWSWIRQHPGKGLEWIGYIYYSG-STNYNPSLKS
6.4.1 : N-QVQLQFSGPGLVRPSETLSLTCTVSGG--SISYYYWNWIRQPAGKGLEWIGRIYTSG-STNYNPSLKS
2345 : N-QVQLQESGPGVVKPSQTLSLTCTVSGGSISSGGYYWSWIRQHPEKGLEWIGYIFYSG-RTYYNPSLKS
2349 : N-QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGYYYWSWIRQLPGKGLEWIGYIYYSG-STYYNPSLKS

CDR3

2250 : KATFTADTSSNTAYMQLSSLTSEDSAVYYCARKN---RN----YYGNYVVWGQGTLVTVSA-C
2253 : KATFTADTSSNTAYMQLSSLTSEDSAVYYCARKN---RN----YYGNYVVWGAGTTLTVSS-C
2256 : KATLTVDKSSSTAYMQLSSLTSEDSAVYYCARKY---YYGNSYRSWYFDVWGAGTTVTVSS-C

6.1.2 : RVTISVDTSKNQFSLKLSSVTAADTAVYYCTRDRDYDSTGDYYSYYGMDVWGQGTTVTVSS-C
6.4.1 : RVTMSVDTSKNQFSLKLSSVTAADTAVYYCARDGGYSNPFD---------WGQGTLVTVSS-C
2345 : RVTISVDTSKNQFSLKLNSVTAADTAVYYCARER-IAAGADYYYNGLDVWGQGTTVTVSS-C
2349 : RITISVDTSKNQFSLKLSSVTAADTAVYYCARER-IAAAGTDYYYNGLAVWGQGTTVTVSS-C

FIG. 9

Light chain sequeces of anti-rkC agonist MAbs

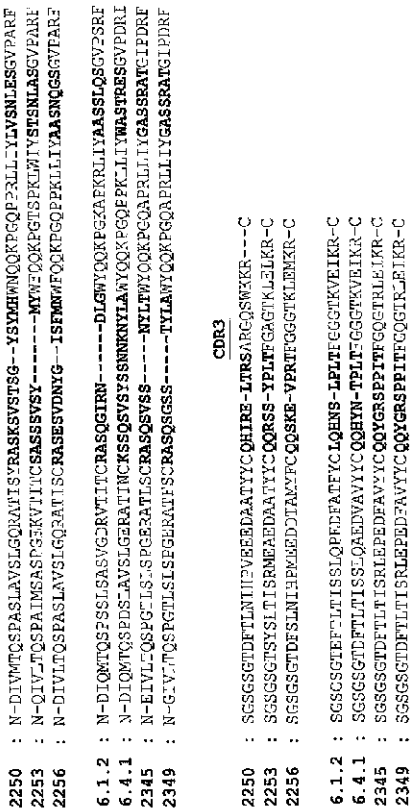


FIG. 10

Antibody — Heavy chains

	Family	CDR1	CDR2	CDR3
Murine				
2250	VHII	FWIEWVK	EILPGSDNTNYNEKFKG	KNRN----YYGNTVV
2253	VHII	FWIEWVK	EILPGSDNTNYNEKFKG	KNRN----YYGNTVV
2256	VHII	YWMHWVK	EIYPSNGRTNYNEKFKS	KYYYGNSYRSWYFDV
Human				
6.1.2	VHII	SGGYYWS	YIYYSGSTNYNPSLKS	DRDYDSTGDYYSYYGMDV
6.4.1	VIII	ISTYYWN	RIYTSGSTNYNPSLKS	DGGYSNPFD---------
2345	VHII	SGGYYWS	YIFYSGRTYYNPSLKS	ER-IAAAGADYYYNGLDV
2349	VHII	SGYYYWS	YIYYSGSTYYNPSLKS	ER-IAAAGTDYYYNGLAV

Antibody — Light chains

	Family	CDR1	CDR2	CDR3
Murine				
2250	V KappaI	RASKSVSTSGYSYMH	LVSNLES	QHIRELTRS
2253	V KappaIV	SASSSVS-----YMY	STSNLAS	QQRSSYPLT
2256	V KappaIII	RASESVDNYGISFMN	AASNQGS	QQSKEVPRT
Human				
6.1.2	V KappaI	RASQGIRN------DLG	AASSLQS	LQHNS-LPLT
6.4.1	V KappaI	KSSQSVSYSSNNKNYLA	WASTRES	QQHYN-TPLT
2345	V KappaIII	RASQSVSS-----NYLT	GASSRAT	QQYGRSPPIT
2349	V KappaIII	RASQSGSS-----TYLA	GASSRAT	QQYGRSPPIT

FIG. 11

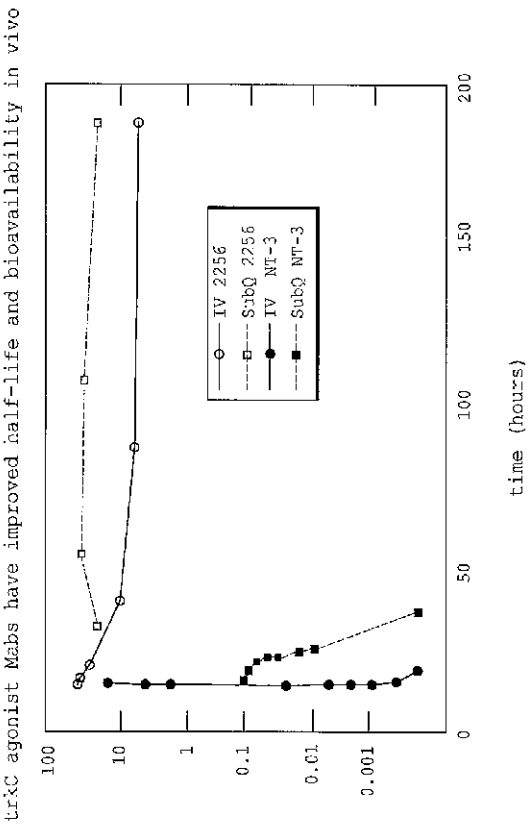

trkC agonist Mabs have improved half-life and bioavailability in vivo
100
10
1
0.1
0.01
0.001
0
50
100
150
200
time (hours)
IV 2256
SubQ 2256
IV NT-3
SubQ NT-3

FIG. 12

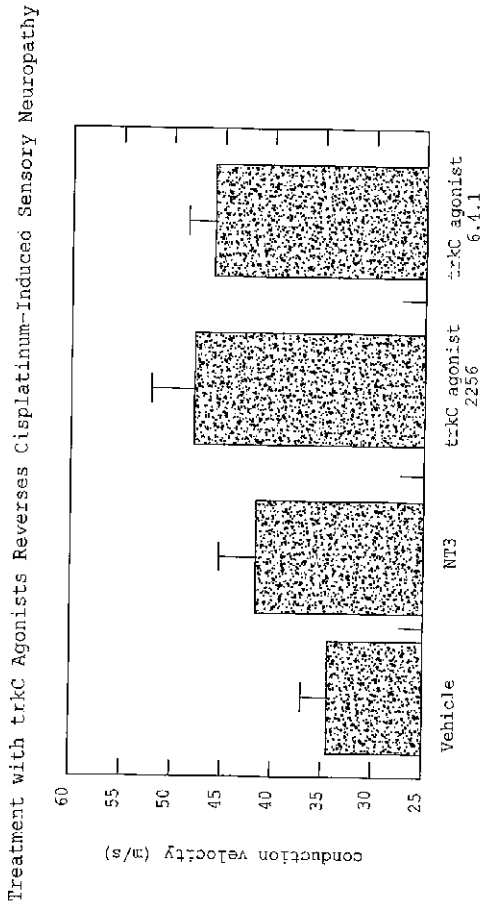

Treatment with trkC Agonists Reverses Cisplatinum-Induced Sensory Neuropathy
conduction velocity (m/s)
60
55
50
45
40
35
30
25
Vehicle
NT3
trkC agonist 2256
trkC agonist 6.4.1

FIG. 13

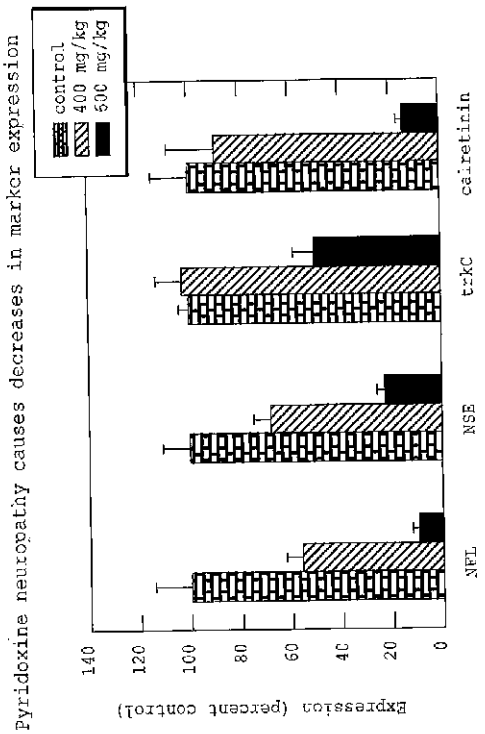

Pyridoxine neuropathy causes decreases in marker expression
control
400 mg/kg
600 mg/kg
Expression (percent control)
140
120
100
80
60
40
20
0
NFL
NSE
t-kC
cairetinin

FIG. 14

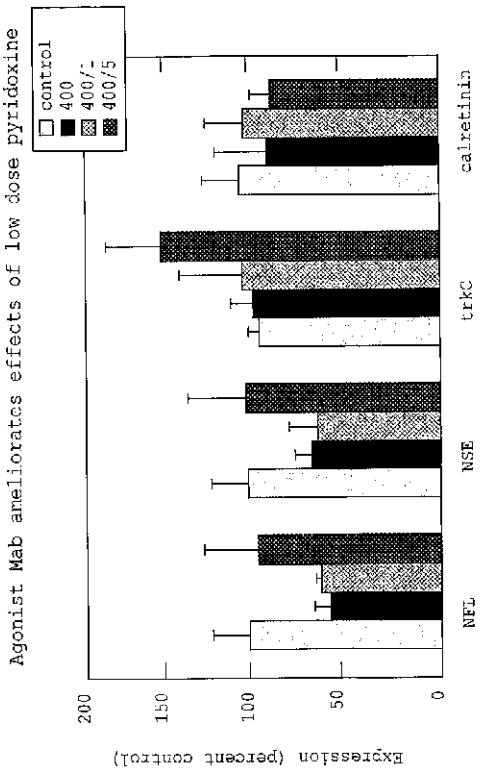

Agonist Mab ameliorates effects of low dose pyridoxine
control
400
400/5
400/5
calretinin
trkC
NSE
NFL
200
150
100
50
0
Expression (percent control)

FIG. 15

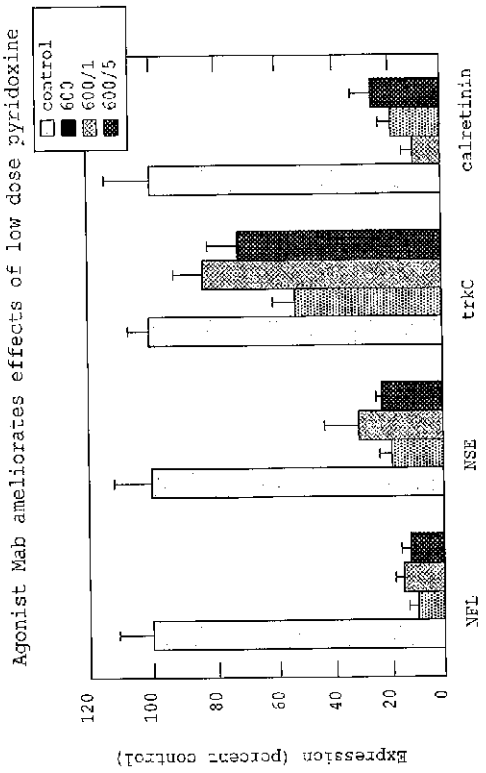

Agonist Mab ameliorates effects of low dose pyridoxine
control
600
600/1
600/5
calretinin
trkC
NSE
NFL
120
100
80
60
40
20
0
Expression (percent control)

FIG. 16

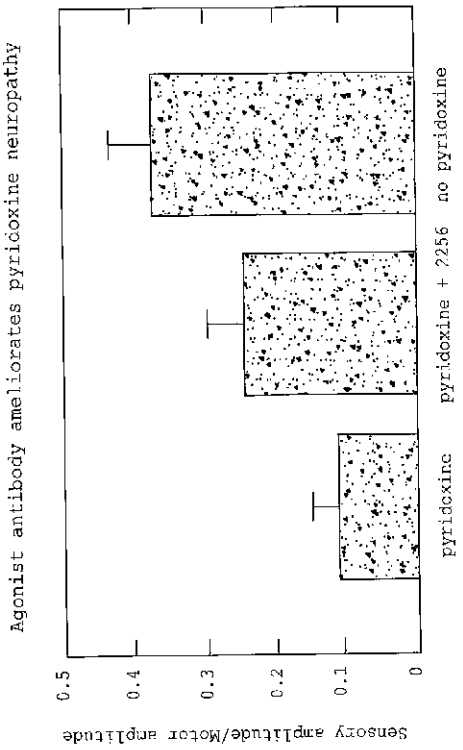

Agonist antibody ameliorates pyridoxine neuropathy
Sensory amplitude/Motor amplitude
0.5
0.4
0.3
0.2
0.1
0
pyridoxine
pyridoxine + ?256
no pyridoxine

FIG. 17

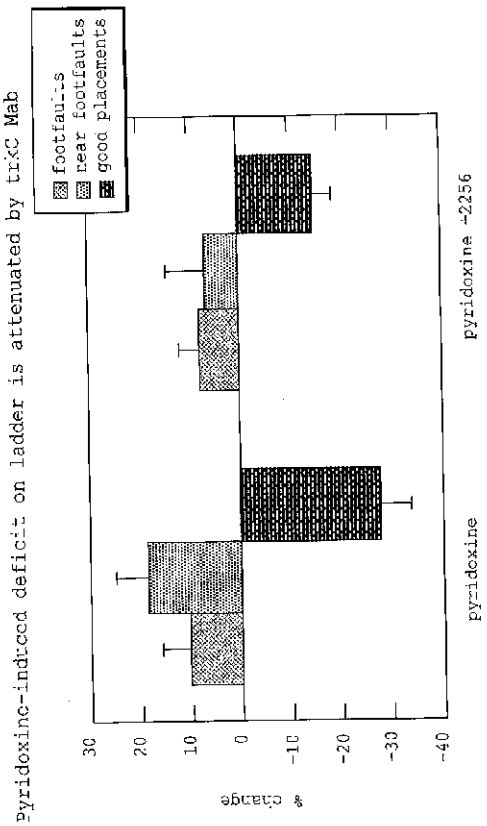

Pyridoxine-induced deficit on ladder is attenuated by trkC Mab
footfaults
rear footfaults
good placements
% change
30
20
10
0
-10
-20
-30
-40
pyridoxine
pyridoxine +2256

FIG. 18

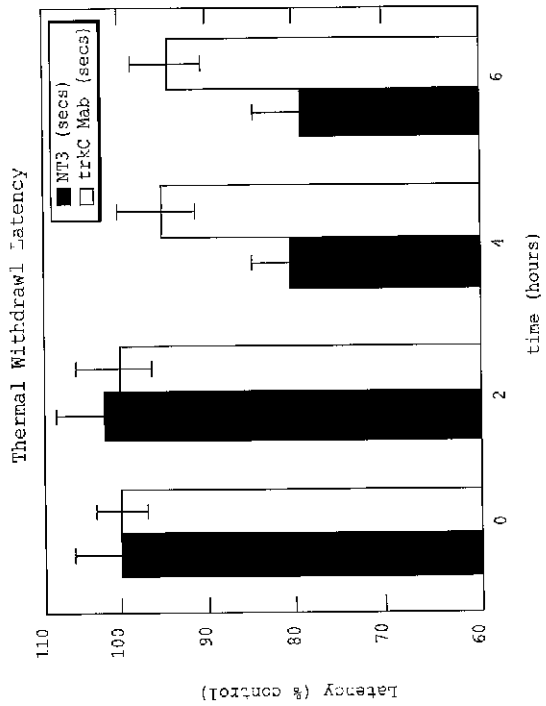

Thermal Withdrawl Latency
NT3 (secs)
trkC Mab (secs)
Latency (% control)
110
100
90
80
70
60
0
2
4
6
time (hours)

FIG. 19

```
1 CPANCVCSXT ZINCRRPDDG NAFPJLEGQD SGMSNGNAXI MITDISRNIT SIELRWRSLI HTLNAVDMSL YYGLQALITK NSSLRSIQRR AFANNPHLRX

101 INLASSNRLTT LSWQLEQTLS IRELQLZQNF FNCSCDIRWM QLKQEQQEAK LNSQNLYCIN ADGSQLPJER MX-SQCDLPP ISVSHVRLTV RGGDMAVITC
                                                                    ^START DOMAIN 4

201 NGSGGRPEDV DWIVVGLQSI NTAQTNIAWT NVLALNITLV NVISELDMGFT LECIAENVVG YSNASVALJV KYPSRVVSLE EZSLRLEHCI ZKVVRGRPPP
                                                                ^ START DOMAIN 5

301 TLEWLFNGQP LRESKIIHYE YYQPGE-SFS CLLFNETPEY NNGNYTLIAK NJLGTANQTI NGHFLKEPFF ESDRAFTLFD EVSPLPPITV THKPSEDTFG
                                                                        END DOMAIN 5^

401 VSIAVGLAAF ACVLLVVLFV MTNRKTGRSK FGMKGIVAVI SGEDDSASPL HEINNHGITTP SSLDAGPDTV VIGMSRIPVT ZNFQYJRQGH WCLKPLTTVQ

501 HIKRRDIVJK KJLGAGAFGK VFLAECYNLS PTKDKVLVAV KALKDPTLAA RGDFQREALL LNNLQHEHIV KFWGVCGDGD FLMVFEYMK HGDLNKFLRA

601 HGPDAMILVD GQPRQAKGEL GLSQMLHIAS QIASGMVYLA SQHFVHRDLA TRNCLVGANL LVKIGDFGMS RDVYSTDYYR LGNRSLMPIR WMSPESIMYG KFTTQSDVWS

701 LPIKWMPPES IMYRKFTTES DVWSYGVILW EIFTYGKQPW FQLSNTEVIE CITQGRVLER PRVCPKEVYD VMLGCWQREP QQRLNIKEIY KILHALGKAT

801 PIYLDLLG
```

FIG. 20

FIG. 21

FIG. 21A | FIG. 21B | FIG. 21C

```
 901 ACGCTCGACT GGCTGCACAA TGGCAGCCT CTGCGGGAGT CCAAGATCAT CCATGTGGAA TACTACCAAG AGCGAGAAAT TTCCGAGGGC TGCCTGCTCT
     TGCGAGCTGA CGGACCTGTT ACCGCTCGGA GACGCCCTCA GGTTCTAGTA GGTACACCTT ATGATGGTTC TCCCTCTCTA AAGAATCCCG ACGGACGAGA

1001 TCAACAAGCC CACCCACTAC AACAATGGCA ACTATACCCT CATTGCCAAA AAGGACTTGG GCACAGCCAA CCAGACCATC AATGGCCACT TCCTCAAGGA
     CGTTACCCAT GCGGTTATAG TTGTAGTGCC TGTATAGTGA AGGTATGTGT ATCTCCTGAC CGCGTCACAA GTGTGCGAGT TGGGGCACCT

1101 GCCCTTTCCA GAGAGCAGGG AIAACTTIAT CTTCTTTGAC GAAGTGACTC CCACACCTCC TATTCACTCTG ACCCACAAAC CAGAAGAAGA CACTTTTGGG
     CGGGAAAGGT CTCTCGTCGC TATTGAAATA GAACAAACTG CTTCACCTAG GGTGTGGAGG ATAATGTGAC TCGGTCTTTG GTCTTCTTCT GTGAAAACCC
                                                                                  ^ END DOMAIN 5

1201 GTACCCATAA CAGTTGGACT TGCTGCTTTT GCCTGTCTCC TGTAGGTGGT TCCCTTCGTC ATGATCAACA AATAATGCTG ACGGTCCAAA TTTGGATGA
     CATAGGTATC GTCAACCTGA ACGACGAAAA CGGACACAGG ACAACCACCA AGACAAGCAG TACTACTTGT TTATACCAGC TGCCAGGTTT AAACCTTACT

1301 AGGGTCCCCT CGCTGTCATC AGTGCTGAGG AGGACTCAGC CAGCCCACTG CACCACATCA ACCACGGCAT CACCACGCCC TCGTCACTGG AYGCCGGGCC
     TCCCAGGGCA CCGACAGTAG TCACGACTCC TCCTCGAGTCG GTCGGGTGAC GTGGTGTAGT TGGTGCCGTA GTGGTCCGGG AGCATGACC TACGGCCCGG

1401 CCACACTGTG GTCATTGGCA TGACTCGCAT CCCTGTCAIT GAGAACCCCC AGTACTTCCG TCAGGGACAC AACTGCCACA AGCCGGACAC GTATGTGCAC
     GGTGTGACAC CAGTAACCGT ACTGAGCGTA GGGACAGTAA CTCTTGGCGG TCATGAAGGC AGTCCCTGTC TTGACGGTGT TCGGCCTGTG CATACACGTG

1501 CACATCAAGA GGACGACAT CGTGCTGAAG CGACAACTCG GTGAGGGAGC CTTTGAAAAG TTCTTCCTGG CCGACTCAGC CCGACCAAGG
     GTGTAATTCT CCTGCTGTA GCACGACTTC GCTGTTGACC CACTCCCTCG GAACCTTTTC CAGAAGGACC GGCTGAGTCG GGCTGGTTCC

1601 ACAAACTGCT TGTGGCTGTG AAGGCCCTGA AGGATCCCAC CCTGCTGCC CGGAAGGATT TCCAGAGGGA GCCGAGCTG CTGACCAACC TGCAGCATGA
     TGTTTGACGA ACACCGACAC TTCCCGGGACT TCCTAGGGTG GGACGGACGG GCCTTCCTAA AGGCTCCCT CGGCTCGAC GAGTCGTTGG ACGTCGTACT

1701 GCACATTGTC AAGTTCTATG GAGTGTGCGG CGATCGGGAC CCCGTCATCA TGCTCTTTGA ATACACTGAG CATGGAGACC TGAATAACTT CCTCAGGGCC
     CGTGTAACAG TTCAAGATAC CTCACACGCC GCTAGCCCTG GGGGAGTAGT ACCAGAAACT TATGTACTTC GTACCTCTGG ACTTATTCAA GGAGTCCCGG
```

FIG. 21B

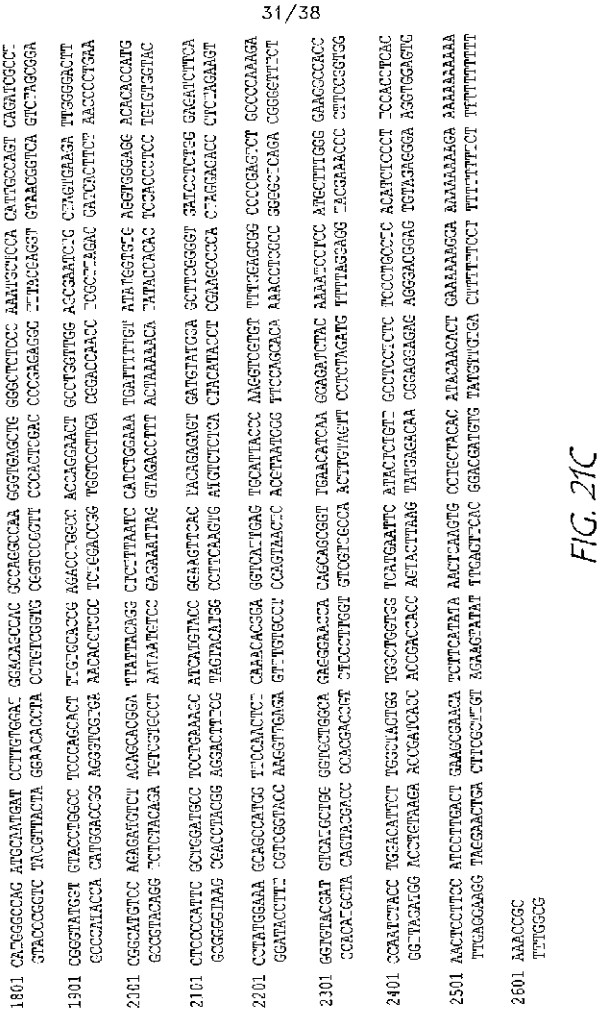


FIG. 21C

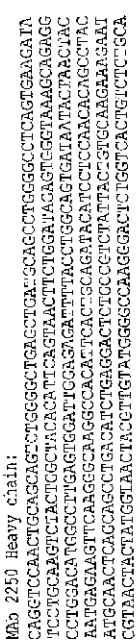


FIG. 22A

FIG. 22B

MAb 2253 Heavy chain:
CAGGTCCAGCTGCAGCAGTCTGGACCTGAGCTGATGCAGCCTGGGGCCTCAGTGAAGATA
TCCTGCAAGTCTACTGGCTACACATTCAGTAACTTCTGGATAGAGTGGGTAAACCAGAGG
CCTGGACATGGCCTTGAGTGGATTGGAGATTTTACCTGGCAGTGGTATAATACCAACTACA
ATGAGAAGTTCAAGGGCAAGGCCACATTCACTGCAGATACATCCTCCAACACAGCCTACA
TGCAACTCAGCACCCTGACATCTGAGGACTCTGCGGTCTATTACTGTGCAAGAAAGAATC
GTAACTACTATAGGTTACTACGTTGTTTGGGGCAGGCACCACTCTCACAGTCTCCTCA

FIG. 23A

MAb 2253 Light chain:
CAAATTGTGCTGACCCAGTCTCCAGCAATCATGTCTGCATCTCCAGGGGAGAAGGTCACC
ATAACCTGCAGTGCCAGCTCAAGTGTAAGTTACATGTACTGGTT
CCAGCAGAAGCCAGGCACTTCTCCCCAAACTCCTGGATTTATAGTACATCCAACCTGGCTTC
TGGAGTCCCTGCTCGCTTCAGTGGCAGTGGATCTGGGACCTCTTACTCTCTCACAATCAG
CCGAATGGAGGCTGAAGATGCTGCCACTTATTACTGCCAGCAAAGGAGTAGTTACCCGCT
CACGTTCGGTGCTGGGACCAAGCTGGAACTAAAACGG

FIG. 23B

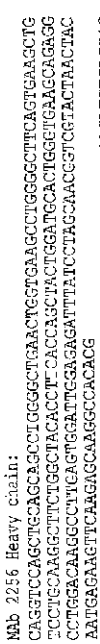


FIG. 24A

FIG. 24B

FIG. 25A

MAb 2345 Heavy chain:
ACCTGCACTGTCTCTGGTGGCTCCATCAGCAGTGGTGGTTACTACTGGAGCTGGATCCGC
CAGCACCCAGGAAAAGGGCCTGGAGTGGTTGGGTACATCTTTTACAGTGGGAGCACCTACT
ACAACCCGTCCCTCAAGAGTCGAGTTACATATCAGTAGACACGTCTAAGAACCAGTTCTC
CCTGAAGCTGAACTCTGTGACAGCCGCGGACACGGCCGTGTATTACTGTGCGAGAGAGCG
GATAGCAGCAGCTGGTGCCGACTACTACTACAAGGGTTTGGACGTCTGGGGCCAAGGGAC
CACGGTCACCGTCTCCTCA

FIG. 25B

MAb 2345 Light chain:
GAAATTGTGTTGACGCAGTCTCCAGCCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACC
CTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAGCAACTACTTAACCTGGTACCAGCAGAAA
CCTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCA
GACAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAG
CCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTATGGTCGCTCACCTCCGATCACCTTC
GGCCAAGGGACACGACTGGAGATTAAACGA

FIG. 26A

MAb 2349 Heavy chain:
CAGGTGCAGCTGCAGGAGTCGGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCCTC
ACCTGCACTGTCTCTGGTGGCTCCATCAGCAGTGGTTACTATTGGAGCTGGATCCGC
CAGCACCCAGGGAAGGGCCTGGAGTGGATTGGGTACATCTATTACAGTGGGAGCACCTAC
TACAACCCGTCCCTCAAGAGTCGACTTACTATCAGTAGACACGTCTAAGAACCAGTTCTC
CCTGAAGCTGAGCTCTGTGACCGCGGCGGACACGGCCGTGTATTACTGTGCGAGAGAGCG
GACAGCAGCACCTGGAACGGACTACTACTACAACGGTTTGGCGTCTGGGCCGTTGGGCAC
CACGGTCACCGTCTCCTCA

FIG. 26B

MAb 2349 Light chain:
GGAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACC
TTCTCCTGCAGGGCCAGTCAGAGTGGTAGCAGCACCTACTTAGCTGGTACCAGCAGAAACC
CTGGCCAGGCTCCCAGGCTCCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAG
ACAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGC
CTGAAGATTTTGCAGTCTATTACTGTCAGCAGTATGGTAGGTCACCTCCGATCACCTTCG
GCCAAGGGACACGACTGGAGATTAAACGA

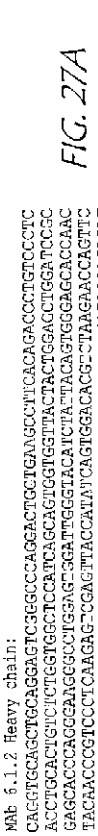


FIG. 27A

FIG. 27B

MAb 6.4.1 Heavy chain:
CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAGGCCTTCGGAGACCCTGTCCCTC
ACCTGCACTGTCTCTGGTGGCTCCATCAGTAGTTACTACTGGAACTGGATCCGGCAGCCCC
GCCGGGAAGGGACTGGAGTGGATTGGGCGTATCTATACCAGTGGCAGCACCAACTACAACC
CCCTCCCTCAAGAGTCGAGTCACCATGTCAGTAGACACGTCCAAGAACCAGTTGTCCCTG
AAGCTGAGCTCTGTGACCGCCGCGGACACGGCCGTCTATTACTGTGCGAGAGATGGGGGC
TACAGTAACCCTTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA

FIG. 28A

MAb 6.4.1 Light chain:
GATATCCAGATGACCCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAGGGCCACC
ATCAACTGCAAGTCCAGCCAGAGTGTTTTATACAGCTCCAACAATAAGAACTACTTAGCT
TGGTACCAGCAGAAACCTGGACAGCCTCCTAAGCTG
CTCATTTACTGGGCATCTACCGGGAATCCGGGGTCCCTGACCGAATCAGTGGCAGCGGG
TCTGGGACAGATTTCACTCTCACCATCAGCAGCCTGCAGGCTGAAGATGTGGCAGTTTAT
TACTGTCAACAACATTATTACTCCACTCACTTTCGGCGGAGGGACCAAGGTGGAGATC
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FIG. 28B

SEQUENCE LISTING

<110> Genentech, Inc.
Devaux, Brigitte
Bongo, Jo-Anne S.
Presta, Leonard G.
Shelton, David L.

<120> AGONIST ANTI-TRK-C MONOCLONAL ANTIBODIES

<130> GENENT.040QPC

<140> 60/238,389
<141> 2000-10-05

<160> 89

<170> FastSEQ for Windows Version 4.0

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Glu Ile Leu Pro Gly Ser Asp Asn Thr Asn Tyr Asn Glu Lys Phe Lys
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Gly

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<211> 17
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<400> 7
Glu Ile Tyr Pro Ser Asn Gly Arg Thr Asn Tyr Asn Glu Lys Phe Lys
1 5 10 15
Ser

<210> 8
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Tyr Ile Tyr Tyr Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys Ser
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1 5 10 15

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<211> 16
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Tyr Ile Phe Tyr Ser Gly Arg Thr Tyr Tyr Asn Pro Ser Leu Lys Ser
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Tyr Ile Tyr Tyr Ser Gly Ser Thr Tyr Tyr Asn Pro Ser Leu Lys Ser
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Lys Asn Arg Asn Tyr Tyr Gly Asn Tyr Val Val
1 5 10

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<400> 13
Lys Tyr Tyr Tyr Gly Asn Ser Tyr Arg Ser Trp Tyr Phe Asp Val
1 5 10 15

<210> 14
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Asp Arg Asp Tyr Asp Ser Thr Gly Asp Tyr Tyr Ser Tyr Tyr Gly Met
1 5 10 15
Asp Val

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Asp Gly Gly Tyr Ser Asn Pro Phe Asp

1 5

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Glu Arg Ile Ala Ala Ala Gly Ala Asp Tyr Tyr Tyr Asn Gly Leu Asp
1 5 10 15
Val

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Glu Arg Ile Ala Ala Ala Gly Thr Asp Tyr Tyr Tyr Asn Gly Leu Ala
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Val

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Arg Ala Ser Lys Ser Val Ser Thr Ser Gly Tyr Ser Tyr Met His
1 5 10 15

<210> 19
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<400> 19
Ser Ala Ser Ser Ser Val Ser Tyr Met Tyr
1 5 10

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Arg Ala Ser Glu Ser Val Asp Asn Tyr Gly Ile Ser Phe Met Asn
1 5 10 15

<210> 21

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Arg Ala Ser Gln Gly Ile Arg Asn Asp Leu Gly
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Lys Ser Ser Gln Ser Val Ser Tyr Ser Ser Asn Asn Lys Asn Tyr Leu
1 5 10 15
Ala

<210> 23
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<213> Homo sapiens

<400> 23
Arg Ala Ser Gln Ser Val Ser Ser Asn Tyr Leu Thr
1 5 10

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<400> 24
Arg Ala Ser Gln Ser Gly Ser Ser Thr Tyr Leu Ala
1 5 10

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<213> Murine

<400> 25
Leu Val Ser Asn Leu Glu Ser
1 5

<210> 26
<211> 7
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<213> Murine

<400> 26
Ser Thr Ser Asn Leu Ala Ser

1 5

<210> 27
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Ala Ala Ser Asn Gln Gly Ser
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Ala Ala Ser Ser Leu Gln Ser
1 5

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<400> 29
Trp Ala Ser Thr Arg Glu Ser
1 5

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Gly Ala Ser Ser Arg Ala Thr
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<400> 31
Gln His Ile Arg Glu Leu Thr Arg Ser
1 5

<210> 32
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Gln Gln Arg Ser Ser Tyr Pro Leu Thr
1 5

<210> 33
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<400> 33
Gln Gln Ser Lys Glu Val Pro Arg Thr
1 5

<210> 34
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<213> Homo sapiens

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Leu Gln His Asn Ser Leu Pro Leu Thr
1 5

<210> 35
<211> 9
<212> PRT
<213> Homo sapiens

<400> 35
Gln Gln His Tyr Asn Thr Pro Leu Thr
1 5

<210> 36
<211> 10
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<213> Homo sapiens

<400> 36
Gln Gln Tyr Gly Arg Ser Pro Pro Ile Thr
1 5 10

<210> 37
<211> 7
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<223> Xaa = F or Y

<221> UNSURE
<222> 3
<223> Xaa = I or M

<221> UNSURE
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<223> Xaa = E or F

<400> 37
Xaa Trp Xaa Xaa Trp Val Lys
1 5

<210> 38
<211> 17
<212> PRT
<213> Murine

<220>
<221> UNSURE
<222> 5
<223> Xaa = L or Y

<221> UNSURE
<222> 6
<223> Xaa = G or S

<221> UNSURE
<222> 6
<223> Xaa = S or N

<221> UNSURE
<222> 7
<223> Xaa = D or G

<221> UNSURE
<222> 8
<223> Xaa = K or R

<221> UNSURE
<222> 17
<223> Xaa = G or S

<400> 38
Glu Ile Xaa Pro Xaa Xaa Xaa Xaa Thr Asn Tyr Asn Glu Lys Phe Lys
1 5 10 15
Xaa

<210> 39
<211> 7
<212> PRT
<213> Homo sapiens

<220>
<221> UNSURE
<222> 1
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<221> UNSURE

<222> 2
<223> Xaa = G or S

<221> UNSURE
<222> 3
<223> Xaa = G, T or Y

<221> UNSURE
<222> 7
<223> Xaa = S or N

<400> 39
Xaa Xaa Xaa Tyr Tyr Trp Xaa
1 5

<210> 40
<211> 16
<212> PRT
<213> Homo sapiens

<220>
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<223> Xaa = Y or R

<221> UNSURE
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<221> UNSURE
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<223> Xaa = Y or T

<221> UNSURE
<222> 8
<223> Xaa = S or R

<221> UNSURE
<222> 10
<223> Xaa = N or Y

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Xaa Ile Xaa Xaa Ser Gly Ser Xaa Thr Xaa Asn Pro Ser Leu Lys Ser
1 5 10 15

<210> 41
<211> 17
<212> PRT
<213> Homo sapiens

<220>
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<223> Xaa = A or T

<221> UNSURE

<222> 16
<223> Xaa = D or A

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Glu Arg Ile Ala Ala Ala Gly Xaa Asp Tyr Tyr Tyr Asn Gly Leu Xaa
1 5 10 15
Val

<210> 42
<211> 122
<212> PRT
<213> Murine

<400> 42
Asn Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Met Gln Pro Gly
1 5 10 15
Ala Ser Val Lys Ile Ser Cys Lys Ser Thr Gly Tyr Thr Phe Ser Asn
20 25 30
Phe Trp Ile Glu Trp Val Lys Gln Arg Pro Gly His Gly Leu Glu Trp
35 40 45
Ile Gly Glu Ile Leu Pro Gly Ser Asp Asn Thr Asn Tyr Asn Glu Lys
50 55 60
Phe Lys Gly Lys Ala Thr Phe Thr Ala Asp Thr Ser Ser Asn Thr Ala
65 70 75 80
Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr
85 90 95
Cys Ala Arg Lys Asn Arg Asn Tyr Tyr Gly Asn Tyr Val Val Trp Gly
100 105 110
Gln Gly Thr Leu Val Thr Val Ser Ala Cys
115 120

<210> 43
<211> 122
<212> PRT
<213> Murine

<400> 43
Asn Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Met Gln Pro Gly
1 5 10 15
Ala Ser Val Lys Ile Ser Cys Lys Ser Thr Gly Tyr Thr Phe Ser Asn
20 25 30
Phe Trp Ile Glu Trp Val Lys Gln Arg Pro Gly His Gly Leu Glu Trp
35 40 45
Ile Gly Glu Ile Leu Pro Gly Ser Asp Asn Thr Asn Tyr Asn Glu Lys
50 55 60
Phe Lys Gly Lys Ala Thr Phe Thr Ala Asp Thr Ser Ser Asn Thr Ala
65 70 75 80
Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr
85 90 95
Cys Ala Arg Lys Asn Arg Asn Tyr Tyr Gly Asn Tyr Val Val Trp Gly
100 105 110
Ala Gly Thr Thr Leu Thr Val Ser Ser Cys
115 120

<210> 44
<211> 126
<212> PRT
<213> Murine

<400> 44
Asn Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Lys Pro Gly
1 5 10 15
Ala Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser
20 25 30
Tyr Trp Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp
35 40 45
Ile Gly Glu Ile Tyr Pro Ser Asn Gly Arg Thr Asn Tyr Asn Glu Lys
50 55 60
Phe Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala
65 70 75 80
Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr
85 90 95
Cys Ala Arg Lys Tyr Tyr Tyr Gly Asn Ser Tyr Arg Ser Trp Tyr Phe
100 105 110
Asp Val Trp Gly Ala Gly Thr Thr Leu Thr Val Ser Ser Cys
115 120 125

<210> 45
<211> 130
<212> PRT
<213> Homo sapiens

<400> 45
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1 5 10 15
Gln Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Ser
20 25 30
Gly Gly Tyr Tyr Trp Ser Trp Ile Arg Gln His Pro Gly Lys Gly Leu
35 40 45
Glu Trp Ile Gly Tyr Ile Tyr Tyr Ser Gly Ser Thr Asn Tyr Asn Pro
50 55 60
Ser Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln
65 70 75 80
Phe Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Thr Arg Asp Arg Asp Tyr Asp Ser Thr Gly Asp Tyr Tyr Ser
100 105 110
Tyr Tyr Gly Met Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser
115 120 125
Ser Cys
130

<210> 46
<211> 119
<212> PRT
<213> Homo sapiens

<400> 46
Asn Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser
1 5 10 15

```
Gln Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Thr
            20                  25                  30
Tyr Tyr Trp Asn Trp Ile Arg Gln Pro Ala Gly Lys Gly Leu Glu Trp
        35                  40                  45
Ile Gly Arg Ile Tyr Thr Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu
    50                  55                  60
Lys Ser Arg Val Thr Met Ser Val Asp Thr Ser Lys Asn Gln Phe Ser
65                  70                  75                  80
Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Arg Asp Gly Gly Tyr Ser Asn Pro Phe Asp Trp Gly Gln Gly Thr
            100                 105                 110
Leu Val Thr Val Ser Ser Cys
        115
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<210> 47
<211> 129
<212> PRT
<213> Homo sapiens

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<400> 47
Asn Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser
 1               5                  10                  15
Gln Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Ser
            20                  25                  30
Gly Gly Tyr Cys Trp Ser Trp Ile Arg Gln His Pro Glu Lys Gly Leu
        35                  40                  45
Glu Trp Ile Gly Tyr Ile Phe Tyr Ser Gly Arg Thr Tyr Cys Asn Pro
    50                  55                  60
Ser Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln
65                  70                  75                  80
Phe Ser Leu Lys Leu Asn Ser Val Thr Ala Ala Asp Thr Ala Val Tyr
                85                  90                  95
Tyr Cys Ala Arg Glu Arg Ile Ala Ala Ala Gly Ala Asp Tyr Tyr Cys
            100                 105                 110
Asn Gly Leu Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
        115                 120                 125
Cys
```

<210> 48
<211> 129
<212> PRT
<213> Homo sapiens

```
<400> 48
Asn Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser
 1               5                  10                  15
Gln Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Ser
            20                  25                  30
Gly Tyr Cys Tyr Trp Ser Trp Ile Arg Gln His Pro Gly Lys Gly Leu
        35                  40                  45
Glu Trp Ile Gly Tyr Ile Tyr Cys Ser Gly Ser Thr Tyr Tyr Asn Pro
    50                  55                  60
Ser Leu Lys Ser Arg Leu Thr Ile Ser Val Asp Thr Ser Lys Asn Glu
65                  70                  75                  80
```

```
Phe Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr
                85                  90                  95
Tyr Cys Ala Arg Glu Arg Ile Ala Ala Ala Gly Thr Asp Tyr Tyr Tyr
            100                 105                 110
Asn Gly Leu Ala Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
        115                 120                 125
Cys
```

<210> 49
<211> 112
<212> PRT
<213> Murine

<400> 49
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Asn Asp Ile Val Met Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu
 1               5                  10                  15
Gly Gln Arg Ala Thr Ile Ser Tyr Arg Ala Ser Lys Ser Val Ser Thr
            20                  25                  30
Ser Gly Tyr Ser Tyr Met His Trp Asn Gln Gln Lys Pro Gly Gln Pro
        35                  40                  45
Pro Arg Leu Leu Ile Tyr Leu Val Ser Asn Leu Glu Ser Gly Val Pro
    50                  55                  60
Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile
65                  70                  75                  80
His Pro Val Glu Glu Glu Asp Ala Ala Thr Tyr Tyr Cys Gln His Ile
                85                  90                  95
Arg Glu Leu Thr Arg Ser Ala Arg Gly Gln Ser Trp Lys Lys Arg Cys
            100                 105                 110
```

<210> 50
<211> 109
<212> PRT
<213> Murine

<400> 50
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Asn Gln Ile Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro
 1               5                  10                  15
Gly Glu Lys Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr
            20                  25                  30
Met Tyr Trp Phe Gln Gln Lys Pro Gly Thr Ser Pro Lys Leu Trp Ile
        35                  40                  45
Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly
    50                  55                  60
Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg Met Glu Ala
65                  70                  75                  80
Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Arg Ser Ser Tyr Pro Leu
                85                  90                  95
Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg Cys
            100                 105
```

<210> 51
<211> 114
<212> PRT
<213> Murine

<400> 51
Asn Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu
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Gly Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Asp Asn
20 25 30
Tyr Gly Ile Ser Phe Met Asn Trp Phe Gln Gln Lys Pro Gly Gln Pro
35 40 45
Pro Lys Leu Leu Ile Tyr Ala Ala Ser Asn Gln Gly Ser Gly Val Pro
50 55 60
Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu Asn Ile
65 70 75 80
His Pro Met Glu Glu Asp Asp Thr Ala Met Tyr Phe Cys Gln Gln Ser
85 90 95
Lys Glu Val Pro Arg Thr Phe Gly Gly Gly Thr Lys Leu Glu Met Lys
100 105 110
Arg Cys

<210> 52
<211> 110
<212> PRT
<213> Homo sapiens

<400> 52
Asn Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15
Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn
20 25 30
Asp Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu
35 40 45
Ile Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser
50 55 60
Gly Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln
65 70 75 80
Pro Glu Asp Phe Ala Thr Phe Tyr Cys Leu Gln His Asn Ser Leu Pro
85 90 95
Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Cys
100 105 110

<210> 53
<211> 116
<212> PRT
<213> Homo sapiens

<400> 53
Asn Asp Ile Gln Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu
1 5 10 15
Gly Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Ser Tyr
20 25 30
Ser Ser Asn Asn Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly
35 40 45
Gln Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly
50 55 60
Val Pro Asp Arg Ile Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu
65 70 75 80

Thr Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln
35 90 95
Gln His Tyr Asn Thr Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu
100 105 110
Ile Lys Arg Cys
115

<210> 54
<211> 112
<212> PRT
<213> Homo sapiens

<400> 54
Asn Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro
1 5 10 15
Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser
20 25 30
Asn Tyr Leu Thr Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu
35 40 45
Leu Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe
50 55 60
Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu
65 70 75 80
Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Arg Ser
85 90 95
Pro Pro Ile Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys Arg Cys
100 105 110

<210> 55
<211> 112
<212> PRT
<213> Homo sapiens

<400> 55
Asn Gly Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro
1 5 10 15
Gly Glu Arg Ala Thr Phe Ser Cys Arg Ala Ser Gln Ser Gly Ser Ser
20 25 30
Thr Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu
35 40 45
Leu Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe
50 55 60
Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu
65 70 75 80
Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Arg Ser
85 90 95
Pro Pro Ile Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys Arg Cys
100 105 110

<210> 56
<211> 808
<212> PRT
<213> Homo sapiens

<400> 56

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Cys Pro Ala Asn Cys Val Cys Ser Lys Thr Glu Ile Asn Cys Arg Arg
 1               5                  10                  15
Pro Asp Asp Gly Asn Leu Phe Pro Leu Leu Glu Gly Gln Asp Ser Gly
            20                  25                  30
Asn Ser Asn Gly Asn Ala Asn Ile Asn Ile Thr Asp Ile Ser Arg Asn
        35                  40                  45
Ile Thr Ser Ile His Ile Glu Asn Trp Arg Ser Leu His Thr Leu Asn
    50                  55                  60
Ala Val Asp Met Glu Leu Tyr Thr Gly Leu Gln Lys Leu Thr Ile Lys
65                  70                  75                  80
Asn Ser Gly Leu Arg Ser Ile Gln Pro Arg Ala Phe Ala Lys Asn Pro
                85                  90                  95
His Leu Arg Tyr Ile Asn Leu Ser Ser Asn Arg Leu Thr Thr Leu Ser
            100                 105                 110
Trp Gln Leu Phe Gln Thr Leu Ser Leu Arg Glu Leu Gln Leu Glu Gln
        115                 120                 125
Asn Phe Phe Asn Cys Ser Cys Asp Ile Arg Trp Met Gln Leu Trp Gln
    130                 135                 140
Glu Gln Gly Glu Ala Lys Leu Asn Ser Gln Asn Leu Tyr Cys Ile Asn
145                 150                 155                 160
Ala Asp Gly Ser Gln Leu Pro Leu Phe Arg Met Asn Ile Ser Gln Cys
                165                 170                 175
Asp Leu Pro Glu Ile Ser Val Ser His Val Asn Leu Thr Val Arg Glu
            180                 185                 190
Gly Asp Asn Ala Val Ile Thr Cys Asn Gly Ser Gly Ser Pro Leu Pro
        195                 200                 205
Asp Val Asp Trp Ile Val Thr Gly Leu Gln Ser Ile Asn Thr His Gln
    210                 215                 220
Thr Asn Leu Asn Trp Thr Asn Val His Ala Ile Asn Leu Thr Leu Val
225                 230                 235                 240
Asn Val Thr Ser Glu Asp Asn Gly Phe Thr Leu Thr Cys Ile Ala Gln
                245                 250                 255
Asn Val Val Gly Met Ser Asn Ala Ser Val Ala Leu Thr Val Tyr Tyr
            260                 265                 270
Pro Pro Arg Val Val Ser Leu Glu Glu Pro Glu Leu Arg Leu Glu His
        275                 280                 285
Cys Ile Glu Phe Val Val Arg Gly Asn Pro Pro Pro Thr Leu His Trp
    290                 295                 300
Leu His Asn Gly Gln Pro Leu Arg Glu Ser Lys Ile Ile His Val Glu
305                 310                 315                 320
Tyr Tyr Gln Glu Gly Glu Ile Ser Glu Gly Cys Leu Leu Phe Asn Lys
                325                 330                 335
Pro Thr His Tyr Asn Asn Gly Asn Tyr Thr Leu Ile Ala Lys Asn Pro
            340                 345                 350
Leu Gly Thr Ala Asn Gln Thr Ile Asn Gly His Phe Leu Lys Glu Pro
        355                 360                 365
Phe Pro Glu Ser Thr Asp Asn Phe Ile Leu Phe Asp Glu Val Ser Pro
    370                 375                 380
Thr Pro Pro Ile Thr Val Thr His Lys Pro Glu Glu Asp Thr Phe Gly
385                 390                 395                 400
Val Ser Ile Ala Val Gly Leu Ala Ala Phe Ala Cys Val Leu Leu Val
                405                 410                 415
Val Leu Phe Val Met Ile Asn Lys Tyr Gly Arg Arg Ser Lys Phe Gly
            420                 425                 430
Met Lys Gly Pro Val Ala Val Ile Ser Gly Glu Glu Asp Ser Ala Ser
        435                 440                 445
Pro Leu Pro His Ile Asn His Gly Ile Thr Thr Pro Ser Ser Leu Asp
    450                 455                 460
```

```
Ala Gly Pro Asp Thr Val Val Ile Gly Met Thr Arg Ile Pro Val Ile
465                 470                 475                 480
Glu Asn Pro Gln Tyr Phe Arg Gln Gly His Asn Cys His Lys Pro Asp
                485                 490                 495
Thr Tyr Val Gln His Ile Lys Arg Arg Asp Ile Val Leu Lys Arg Glu
            500                 505                 510
Leu Gly Glu Gly Ala Phe Gly Lys Val Phe Leu Ala Glu Cys Tyr Asn
        515                 520                 525
Leu Ser Pro Thr Lys Asp Lys Met Leu Val Ala Val Lys Ala Leu Lys
    530                 535                 540
Asp Pro Thr Leu Ala Ala Arg Lys Asp Phe Gln Arg Glu Ala Glu Leu
545                 550                 555                 560
Leu Thr Asn Leu Gln His Glu His Ile Val Lys Phe Tyr Gly Val Cys
                565                 570                 575
Gly Asp Gly Asp Pro Leu Ile Met Val Phe Glu Tyr Met Lys His Gly
            580                 585                 590
Asp Leu Asn Lys Phe Leu Arg Ala His Gly Pro Asp Ala Met Ile Leu
        595                 600                 605
Val Asp Gly Gln Pro Arg Gln Ala Lys Gly Glu Leu Gly Leu Ser Gln
    610                 615                 620
Met Leu His Ile Ala Ser Gln Ile Ala Ser Gly Met Val Tyr Leu Ala
625                 630                 635                 640
Ser Gln His Phe Val His Arg Asp Leu Ala Thr Arg Asn Cys Leu Val
                645                 650                 655
Gly Ala Asn Leu Leu Val Lys Ile Gly Asp Phe Gly Met Ser Arg Asp
            660                 665                 670
Val Tyr Ser Thr Asp Tyr Tyr Arg Leu Phe Asn Pro Ser Gly Asn Asp
        675                 680                 685
Phe Cys Ile Trp Cys Glu Val Gly Gly His Thr Met Leu Pro Ile Arg
    690                 695                 700
Trp Met Pro Pro Glu Ser Ile Met Tyr Arg Lys Phe Thr Thr Glu Ser
705                 710                 715                 720
Asp Val Trp Ser Phe Gly Val Ile Leu Trp Glu Ile Phe Thr Tyr Gly
                725                 730                 735
Lys Gln Pro Trp Phe Gln Leu Ser Asn Thr Glu Val Ile Glu Cys Ile
            740                 745                 750
Thr Gln Gly Arg Val Leu Glu Arg Pro Arg Val Cys Pro Lys Glu Val
        755                 760                 765
Tyr Asp Val Met Leu Gly Cys Trp Gln Arg Glu Pro Gln Gln Arg Leu
    770                 775                 780
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【国際調査報告】

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 01/20153

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/13 C07K16/28 C12N5/10 C12N15/63 A61K39/395
A61P25/00 A61P25/28 A61P9/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, EMBASE, WPI Data, PAJ, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 97 21732 A (MCGILL UNIVERSITY) 19 June 1997 (1997-06-19) examples claims ---	1-69
A	WO 98 49308 A (GENENTECH INC.) 5 November 1998 (1998-11-05) examples claims --- -/---	1-69

[X] Further documents are listed in the continuation of box C. [X] Patent family members are listed in annex.

* Special categories of cited documents :
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 February 2002	01/03/2002
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Nooij, F

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/20153

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of documents, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	R. URFER ET AL.: "High resolution mapping of the binding site of TrkA for nerve growth factor and TrkC for neurotrophin-3 on the second immunoglobulin-like domain of the Trk receptors." THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 273, no. 10, 6 March 1998 (1998-03-06), pages 5829-5840, XP002116515 Baltimore, MD, USA page 5835, left-hand column, line 31 -right-hand column, line 14 table IV	1-69
A	M. MENDEZ ET AL.: "Functional transplant of megabase human immunoglobulin loci recapitulates human antibody response in mice." NATURE GENETICS, vol. 15, no. 2, February 1997 (1997-02), pages 146-156, XP002067603 New York, NY, USA cited in the application abstract	1-69
T	J. HONGO ET AL.: "Agonist monoclonal antibodies to human trkC receptor map to epitopes overlapping with NT-3 binding site." SOCIETY FOR NEUROSCIENCE ABSTRACTS, vol. 27, no. 1, 2001, page 356 XP001053704 abstract 137.4	1-69

1

Form PCT/ISA/210 (continuation of second sheet) (July 1998)

International Application No. PCT/US 01/20153

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 50-57 and 59-63 (all completely) and claims 58 and 64 (both partially, as far as an in vivo method is concerned) are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

International Application No. PCT/US 01 20153

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-11, 27-48, 50-57, 59-69 (all completely), 21-26, 49, 58 (all partially)

Agonist monoclonal antibody against trkC, its encoding nucleic acid, its manufacture, and its therapeutic use.

2. Claims: 12-20 (all completely), 21-26, 49, 58 (all partially)

Antibodies against trkC with specified CDR amino acid sequences.

INTERNATIONAL SEARCH REPORT

...formation on patent family members

International Application No
PCT/US 01/20153

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9721732	A	19-06-1997	AU	7689496 A	03-07-1997
			WO	9721732 A1	19-06-1997
			EP	0869976 A1	14-10-1998
WO 9849308	A	05-11-1998	US	6333310 B1	25-12-2001
			AU	7154698 A	24-11-1998
			EP	0977860 A1	09-02-2000
			JP	2001527402 T	25-12-2001
			WO	9849308 A1	05-11-1998
			ZA	9803289 A	20-10-1999

Form PCT/ISA/210 (patent family annex) (July 1992)

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(51)Int.Cl.[7]	FI		テーマコード（参考）
Ａ６１Ｐ 25/28	Ａ６１Ｐ 25/28		４Ｈ０４５
Ｃ０７Ｋ 16/28	Ｃ０７Ｋ 16/28		
Ｃ１２Ｎ 5/10	Ｃ１２Ｎ 15/00	Ｃ	
Ｃ１２Ｎ 15/02	Ｃ１２Ｎ 5/00	Ｂ	
// Ｃ１２Ｐ 21/08	Ｃ１２Ｐ 21/08		

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