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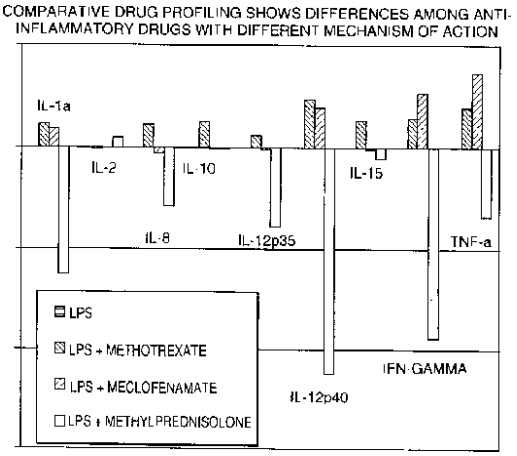
(54)【発明の名称】較正された遺伝子発現プロファイルを使用する生物学的状態又は作用因子を特徴付けするシステム及び方法

(57)【要約】
本発明は、被験体の生物学的状態を評価する方法であって、
ａ．被験体からＲＮＡ類及び蛋白質類の少なくとも１つを有するサンプルを得ること、ｂ．複数の構成要素を含む第１のプロファイルデータセットであって、各々の構成要素が、構成成分の測定が生物学的状態の測定を可能にするように選択された構成成分のパネル中のはっきり識別できるＲＮＡ又は蛋白質構成成分の量の定量的測定値である、第１のプロファイルデータセットをサンプルから得ること、及びｃ．パネルに対する較正されたプロファイルデータセットであって、当該較正されたプロファイルデータセットの各々の構成要素が第１のプロファイルデータセットの対応する構成要素及びパネルに対する基準プロファイルデータセットの対応する構成要素の関数であり、較正されたプロファイルデータセットは被験体の生物学的状態の目安を提供する、パネルに対する較正されたプロファイルデータセットを作ること、を含む方法である。
【選択図】図１２



【特許請求の範囲】
【請求項１】
被験体の生物学的状態を評価する方法であって、
ａ．被験体からＲＮＡ類及び蛋白質類の少なくとも１つを有するサンプルを得ること、
ｂ．複数の構成要素を含む第１のプロファイルデータセットであって、各々の構成要素が、構成成分の測定が生物学的状態の測定を可能にするように選択された構成成分のパネル中のはっきり識別できるＲＮＡ又は蛋白質構成成分の量の定量的測定値である、第１のプロファイルデータセットをサンプルから得ること、及び
ｃ．パネルに対する較正されたプロファイルデータセットであって、当該較正されたプロファイルデータセットの各々の構成要素が第１のプロファイルデータセットの対応する構成要素及びパネルに対する基準プロファイルデータセットの対応する構成要素の関数であり、較正されたプロファイルデータセットは被験体の生物学的状態の目安を提供する、パネルに対する較正されたプロファイルデータセットを作ること、
を含む方法。
【請求項２】
被験体の生物学的状態を評価する方法であって、
ａ．被験体から液体、細胞、及び活性作用因子の少なくとも１つを有する第１のサンプルを得ること、
ｂ．第１のサンプル又はその一部を指標細胞の定義された集団に加えること、
ｃ．指標細胞からＲＮＡ類又は蛋白質類の少なくとも１つを含む第２のサンプルを得ること、
ｄ．複数の構成要素を含む第１のプロファイルデータセットであって、各々の構成要素が、構成成分の測定が生物学的状態の測定を可能にするように選択された構成成分のパネル中のはっきり識別できるＲＮＡ又は蛋白質構成成分の量の定量的測定値である、第１のプロファイルデータセットを第２のサンプルから得ること、及び
ｅ．パネルに対する較正されたプロファイルデータセットであって、当該較正されたプロファイルデータセットの各々の構成要素が第１のプロファイルデータセットの対応する構成要素及びパネルに対する基準プロファイルデータセットの対応する構成要素の関数であり、較正されたプロファイルデータセットは被験体の生物学的状態の目安を提供する、パネルに対する較正されたプロファイルデータセットを作ること、
を含む方法。
【請求項３】
作用因子によって影響される生物学的状態を評価する方法であって、
ａ．作用因子が投与されている細胞の標的集団から、ＲＮＡ類及び蛋白質類の少なくとも１つを有するサンプルを得ること、
ｂ．複数の構成要素を含む第１のプロファイルデータセットであって、各々の構成要素が、構成成分の測定が生物学的状態の測定を可能にするように選択された構成成分のパネル中のはっきり識別できるＲＮＡ又は蛋白質構成成分の量の定量的測定値である、第１のプロファイルデータセットをサンプルから得ること、及び
ｃ．パネルに対する較正されたプロファイルデータセットであって、当該較正されたプロファイルデータセットの各々の構成要素が第１のプロファイルデータセットの対応する構成要素及びパネルに対する基準プロファイルデータセットの対応する構成要素の関数であり、較正されたプロファイルデータセットは作用因子によって影響される生物学的状態の目安を提供する、パネルに対する較正されたプロファイルデータセットを作ること、
を含む方法。
【請求項４】
基準プロファイルデータセットが、当該サンプルのものとは異なる条件下に取られた同じ被験体からの１以上のその他のサンプルから得られる、請求項１乃至２のいずれか１請求項の方法。
【請求項５】

条件が、（ｉ）所定のサンプルが取られる時間、（ｉｉ）所定のサンプルが取られる場所、（ｉｉｉ）所定のサンプルが取られるときの被験体の生理学的な状態、から成る群から選択される、請求項４の方法。

【請求項６】

１以上のその他のサンプルが、最初のサンプルと当該サンプルの間に少なくとも１２ヶ月間あるような期間にわたって取られる、請求項４の方法。

【請求項７】

１以上のその他のサンプルが、最初のサンプルと当該サンプルの間に少なくとも１ヶ月間あるような期間にわたって取られる、請求項４の方法。

【請求項８】

当該サンプルが血液から得られ、そして基準プロファイルデータセットが血液以外の被験体の組織又は体液から得られる、請求項１乃至３のいずれか１請求項の方法。

【請求項９】

基準プロファイルデータセットが、年齢、食餌、投薬、及び環境への暴露の少なくとも１つに関して、当該サンプルが取られたときの被験体の生理学的状態と異なる生理学的状態に被験体があるときに取られた、同じ被験体からの１つ以上のその他のサンプルから得られる、請求項４の方法。

【請求項１０】

基準プロファイルデータセットが、当該サンプルのものと異なる条件下に取られた同じ集団からの１つ以上のその他のサンプルから得られる、請求項３の方法。

【請求項１１】

条件が、（ｉ）所定のサンプルが取られる時間、及び（ｉｉ）所定のサンプルが取られるときの集団の生理学的状態、から成る群から選択される請求項１０の方法。

【請求項１２】

１以上のその他のサンプルが、最初のサンプルと当該サンプルの間に少なくとも１２ヶ月間あるような期間にわたって取られる、請求項１０の方法。

【請求項１３】

１以上のその他のサンプルが、最初のサンプルと当該サンプルの間に少なくとも１ヶ月間あるような期間にわたって取られる、請求項１０の方法。

【請求項１４】

サンプルが血液から得られ、そして基準プロファイルデータセットが血液以外の被験体の組織又は体液から得られる、請求項１０の方法。

【請求項１５】

基準プロファイルデータセットが、年齢、食餌、投薬、及び環境への暴露の少なくとも１つに関して、サンプルが取られたときの被験体の生理学的状態と異なる生理学的に状態に被験体があるときに取られた、共通の被験体に関係する細胞集団の１以上のその他のサンプルから得られる、請求項１０の方法。

【請求項１６】

基準プロファイルデータセットが、１以上の異なる被験体からの１以上のその他のサンプルから得られる、請求項１乃至２のいずれか１請求項の方法。

【請求項１７】

１以上の異なる被験体が、年齢グループ、性別、民族性、地理的位置、食餌、医学的不調、臨床的指標、投薬、身体活動、体重、及び環境への暴露の少なくとも１つの点で被験体と同じである、請求項１６の方法。

【請求項１８】

基準プロファイルデータセットが、異なる複数の被験体に関連する１以上の細胞集団からの１以上のその他のサンプルから得られる、請求項３の方法。

【請求項１９】

１以上の異なる被験体が、年齢グループ、性別、民族性、地理的位置、食餌、医学的不調、臨床的指標、投薬、身体活動、体重、及び環境への暴露の少なくとも１つの点で被験体

と同じである、請求項１８の方法。
【請求項２０】
少なくとも１つのその他の臨床的指標の背景において較正されたプロファイルデータセットを解釈することをさらに含む、請求項１乃至３のいずれか１請求項の方法。
【請求項２１】
指標が、血液の化学的性質、尿分析、Ｘ線、その他の化学的分析、及び身体的所見から成る群から選択される、請求項２０の方法。
【請求項２２】
生物学的状態が、複数の遺伝子が関与する複雑な疾病プロセスであり、当該疾病が、炎症、自己免疫疾患、変性疾患、アレルギー、血管の疾病、局所貧血、癌、発達疾患、ホルモン状態、老化、及び伝染性疾患の少なくとも１つを含むタイプのものである、請求項１乃至３のいずれか１請求項の方法。
【請求項２３】
生物学的状態が、関節炎、喘息、多発性硬化症、及びペリメノポーザル変化（ｐｅｒｉｍｅｎｏｐａｕｓａｌ　ｃｈａｎｇｅ）の１つである、請求項２２の方法。
【請求項２４】
被験体が生きている生物である、請求項１乃至２のいずれか１請求項の方法。
【請求項２５】
被験体が哺乳類である、請求項２４の方法。
【請求項２６】
細胞の集団がヒトの細胞である、請求項３の方法。
【請求項２７】
細胞の集団が哺乳類の細胞である、請求項３の方法。
【請求項２８】
当該サンプルが体液及び組織の１以上から得られる、請求項１乃至３のいずれか１請求項の方法。
【請求項２９】
当該サンプルが血液から得られる、請求項１乃至３のいずれか１請求項の方法。
【請求項３０】
当該サンプルが、バイオプシー、注射針吸引（ｎｅｅｄｌｅ　ａｓｐｉｒａｔｅ）、洗浄サンプル、こすり（ｓｃｒａｐｉｎｇ）、及び外科手術サンプルの１つから得られる、請求項１乃至３のいずれか１請求項の方法。
【請求項３１】
当該サンプルが、状態が臨床的に明らかにされているものと異なるタイプの組織又は液体から得られる、請求項１乃至３のいずれか１請求項の方法。
【請求項３２】
状態が疾病であり、そして当該サンプルが当該疾病の主要な標的であるものと異なるタイプの組織又は液体から得られる、請求項１乃至３のいずれか１請求項の方法。
【請求項３３】
関数が、単純な差以外のものである、請求項１乃至３のいずれか１請求項の方法。
【請求項３４】
関数が、第１のプロファイルデータセットの対応する構成要素の基準プロファイルデータセットの対応する構成要素に対する比の第２の関数である、請求項３３の方法。
【請求項３５】
関数が対数関数である、請求項３４の方法。
【請求項３６】
較正されたプロファイルデータセットの各々の構成要素が、類似の条件下に被験体から取られた類似のサンプルに関して再現可能である、請求項１乃至３のいずれか１請求項の方法。
【請求項３７】

較正されたプロファイルデータセットの各々の構成要素が、類似の条件下に被験体から取られた類似のサンプルに関して１桁の大きさの範囲内で再現可能である、請求項１乃至３のいずれか１請求項の方法。
【請求項３８】
較正されたプロファイルデータセットの各々の構成要素が、類似の条件下に被験体から取られた類似のサンプルに関して５０％以内で再現可能である、請求項１乃至３のいずれか１請求項の方法。
【請求項３９】
較正されたプロファイルデータセットの各々の構成要素が、類似の条件下に被験体から取られた類似のサンプルに関して２０％以内で再現可能である、請求項１乃至３のいずれか１請求項の方法。
【請求項４０】
較正されたプロファイルデータセットの各々の構成要素が、類似の条件下に被験体から取られた類似のサンプルに関して１桁の大きさの範囲内で再現可能である、請求項３４の方法。
【請求項４１】
較正されたプロファイルデータセットの各々の構成要素が、類似の条件下に被験体から取られた類似のサンプルに関して５０％以内で再現可能である、請求項３４の方法。
【請求項４２】
較正されたプロファイルデータセットの各々の構成要素が、類似の条件下に被験体から取られた類似のサンプルに関して２０％以内で再現可能である、請求項３４の方法。
【請求項４３】
較正されたプロファイルデータセットの各々の構成要素が、量Ｄ（ここで、Ｄ＝Ｆ（１．１）－Ｆ（．９）であり、そしてＦは当該第２の関数である）より大きい量だけ異なる値を有する場合、生物学的有意性を有する、請求項３４の方法。
【請求項４４】
生物学的状態が器官に関し、そして構成成分のパネルが当該器官に関係する状態の測定を可能にする、請求項１乃至３のいずれか１請求項の方法。
【請求項４５】
生物学的状態が被験体の系に関し、当該系が、呼吸系、血管系、神経系、代謝系、泌尿系、生殖系、構造系、及び免疫系から成る群から選択され、そして構成成分のパネルが当該系に関係する被験体の状態の測定を可能にする、請求項１乃至２のいずれか１請求項の方法。
【請求項４６】
細胞の集団が被験体から得られ、そして生物学的状態が被験体の系に関し、当該系が、呼吸系、血管系、神経系、代謝系、泌尿系、生殖系、構造系、及び免疫系から成る群から選択され、そして構成成分のパネルが当該系に関係する被験体の状態の測定を可能にする、請求項３の方法。
【請求項４７】
パネルが、炎症パネルの構成成分の少なくとも半分を含む、請求項４６の方法。
【請求項４８】
パネルが、炎症パネルの構成成分の少なくとも８０％を含む、請求項４６の方法。
【請求項４９】
パネルが、細胞成長及び分化パネルの構成成分の少なくとも半分を含む、請求項４６の方法。
【請求項５０】
パネルが、細胞成長及び分化パネルの構成成分の少なくとも８０％を含む、請求項４６の方法。
【請求項５１】
パネルが、毒性パネルの構成成分の少なくとも半分を含む、請求項４６の方法。

【請求項５２】
パネルが、毒性パネルの構成成分の少なくとも８０％を含む、請求項４６の方法。
【請求項５３】
パネル中の構成成分の数が、少なくとも３であるが、１００未満である、請求項１乃至３のいずれか１請求項の方法。
【請求項５４】
パネル中の構成成分の数が、少なくとも４であるが、１００未満である、請求項１乃至３のいずれか１請求項の方法。
【請求項５５】
パネル中の構成成分の数が、少なくとも５であるが、１００未満である、請求項１乃至３のいずれか１請求項の方法。
【請求項５６】
パネル中の構成成分の数が少なくとも６である、請求項１乃至３のいずれか１請求項の方法。
【請求項５７】
作用因子が、薬剤、化合物の混合物、機能性食品、栄養物、治療剤、アレルゲン、及び毒素から成る群から選択される、請求項３の方法。
【請求項５８】
当該サンプルからの第１のプロファイルデータセットの取得が、当該サンプルを核酸プローブのセットとハイブリッド化することを含む、請求項１乃至３のいずれか１請求項の方法。
【請求項５９】
プローブが不溶性マトリックスに結合され、そして当該サンプルがマトリックスに加えられる、請求項５８の方法。
【請求項６０】
作用因子によって影響される状態の評価が、当該作用因子と細胞の集団に投与される第２の作用因子との相互作用を評価することを含む、請求項３の方法。
【請求項６１】
相互作用が中立である、請求項６０の方法。
【請求項６２】
相互作用が干渉である、請求項６０の方法。
【請求項６３】
相互作用が累積的である、請求項６０の方法。
【請求項６４】
相互作用が相乗的である、請求項６０の方法。
【請求項６５】
作用因子が製薬である、請求項６０の方法。
【請求項６６】
第２の作用因子による影響に関連して第１の作用因子による生物学的状態に対する影響を評価する方法であって、
ａ．第１及び第２の作用因子がそれぞれ投与されている第１及び第２の細胞の標的集団から、ＲＮＡ類及び蛋白質類の少なくとも１つを各々が有する第１及び第２のサンプルをそれぞれ得ること、
ｂ．第１のサンプルから第１のプロファイルデータセットをそして第２のサンプルから第２のプロファイルデータセットを得ること、ここで当該プロファイルデータセットの各々が複数の構成要素を含み、各々の構成要素が、構成成分の測定が生物学的状態の測定を可能にするように選択された構成成分のパネル中のはっきり識別できるＲＮＡ又は蛋白質構成成分の量の定量的測定値であるものであること、及び
ｃ．パネルに対する第１の較正されたプロファイルデータセット及び第２のプロファイルデータセットを作ること、ここで、（ｉ）第１の較正されたプロファイルデータセットの

各々の構成要素は第１のプロファイルデータセットの対応する構成要素及びパネルに対する第１の基準プロファイルデータセットの対応する構成要素の関数であり、そして（ｉｉ）第２の較正されたプロファイルデータセットの各々の構成要素は第２のプロファイルデータセットの対応する構成要素及びパネルに対する第２の基準プロファイルデータセットの対応する構成要素の関数であり、これらの較正されたプロファイルデータセットは、第２の作用因子による影響に関連して第１の作用因子による生物学的状態への影響の目安を提供すること、
を含む方法。
【請求項６７】
第１の作用因子が薬剤であり、第２の作用因子が複合混合物である、請求項６６の方法。
【請求項６８】
第１の作用因子が薬剤であり、第２の作用因子が栄養物である、請求項６６の方法。
【請求項６９】
サンプルの取得及び第１のプロファイルデータセットの定量化が、第１の場所において行われ、そして較正されたプロファイルデータセットの作成が、第２の場所においてデジタル記憶媒体上に記憶されたデータベースにアクセスするためにネットワークを使用することを含む、請求項１乃至３のいずれか１請求項の方法。
【請求項７０】
サンプルから定量化された第１のプロファイルデータセットを反映させるためにデータベースをアップデートすることをさらに含む、請求項６９の方法。
【請求項７１】
ネットワークの使用が、世界的なコンピューターネットワークにアクセスすることを含む、請求項６９の方法。
【請求項７２】
作用因子の臨床試験を行う方法であって、
ａ．プラシーボ及び作用因子の選択された一方を被験体のプールの各々の候補にブラインド投与すること、及び
ｂ．そのような投与の影響を監視するために定量的遺伝子発現を使用すること、
を含む方法。
【請求項７３】
被験体のプールが、作用因子への反応を示しそうである候補を識別するために、複数の候補に対して定量的遺伝子発現分析を使用して選択される、請求項７２の方法。
【請求項７４】
投与が、定量的遺伝子発現分析を使用することによって、投与量及び投与量範囲の少なくとも１つを決定することを含む、請求項７２の方法。
【請求項７５】
作用因子の効能及び毒性の少なくとも１つの決定において補助するために定量的遺伝子発現分析を使用することをさらに含む、請求項７２の方法。
【請求項７６】
定量的遺伝子発現分析の使用が、請求項１、２、及び３の少なくとも１つの方法を使用することを含む、請求項７２乃至７５のいずれか１請求項の方法。
【請求項７７】
コンピューター読み取り可能な較正されたプロファイルデータセットが記憶されているデジタル記憶媒体であって、
ａ．較正されたプロファイルデータセットが、作用因子が投与されている標的細胞集団から得られるＲＮＡ類及び蛋白質類の少なくとも１つを有するサンプルに関し、そして
ｂ．較正されたプロファイルデータセットが、第１の複数の構成要素を含み、各々の構成要素が、構成成分の測定が作用因子の投与によって影響される生物学的状態の測定を可能にするように選択された構成成分のパネル中のはっきり識別できるＲＮＡ又は蛋白質構成成分の量の変化の定量的測定値である、

デジタル記憶媒体。
【請求項７８】
（ｉ）較正されたプロファイルデータセットの各々の構成要素が投与後のデータセットの対応する構成要素及び基準データセットの対応する構成要素の関数であり、（ｉｉ）基準データセットの各々の構成要素が、標準条件下のパネル中のはっきり識別できるＲＮＡ又は蛋白質構成成分の量の定量的測定値であり、そして（ｉｉｉ）投与後のデータセットの各々の構成要素が、標的細胞集団への作用因子の投与後の条件下のパネル中のはっきり識別できるＲＮＡ又は蛋白質構成成分の量の定量的測定値である、請求項７７のデジタル記憶媒体。
【請求項７９】
関数が、基準データセットの対応する構成要素の投与後のデータセットの対応する構成要素に対する比の第２の関数である、請求項７８の媒体。
【請求項８０】
第２の関数が対数関数である、請求項７９の媒体。
【請求項８１】
作用因子が製薬である、請求項７７乃至８０のいずれか１請求項のデジタル記憶媒体。
【請求項８２】
作用因子が第２の複数の成分を含む、請求項７７乃至８０のいずれか１請求項のデジタル記憶媒体。
【請求項８３】
作用因子が栄養物である、請求項７７乃至８０のいずれか１請求項のデジタル記憶媒体。
【請求項８４】
第１の複数の構成要素が少なくとも３であるが、１０００未満である、請求項７７乃至８０のいずれか１請求項のデジタル記憶媒体。
【請求項８５】
第１の複数の構成要素が少なくとも４であるが、１０００未満である、請求項７７乃至８０のいずれか１請求項のデジタル記憶媒体。
【請求項８６】
第１の複数の構成要素が少なくとも５であるが、１０００未満である、請求項７７乃至８０のいずれか１請求項のデジタル記憶媒体。
【請求項８７】
第１の複数の構成要素が少なくとも６である、請求項７７乃至８０のいずれか１請求項のデジタル記憶媒体。
【請求項８８】
被験体の集団に関する複数の記録R_iが記憶されているデジタル記憶媒体であって、各々の記録R_iは、コンピューター読み取り可能なプロファイルデータセットＰのはっきり識別できる事実P_iに対応し、ここで、
ａ．プロファイルデータセットＰの各々の事実P_iは被験体から得られるはっきり識別できるサンプルに関係し、当該サンプルはＲＮＡ類及び蛋白質類の少なくとも１つを有し、
ｂ．プロファイルデータセットＰは複数の構成要素M_jを含み、各々の構成要素M_jは、構成成分の測定が生物学的状態の測定を可能にするように選択された構成成分のパネル中のはっきり識別できるＲＮＡ又は蛋白質構成成分の量の定量的測定値であり、
ｃ．各々の記録R_iは、プロファイルデータセットＰの対応するはっきり識別できる事実P_iの各々の構成要素M_{ij}に関して、構成要素M_{ij}の値に対応する値を含み、及び
ｄ．各々の記録R_iは、また、当該記録に関して被験体の特徴への参照を含み、当該特徴は、年齢グループ、性別、民族性、地理的位置、食餌、医学的不調、臨床的指標、投薬、身体活動、体重、及び環境への暴露の少なくとも１つである、
デジタル記憶媒体。
【請求項８９】
各々のサンプルが、作用因子が投与されている標的細胞集団から得られ、そのような標的

細胞集団は被験体から得られたものである、請求項８８のデジタル記憶媒体。
【請求項９０】
多数のコンピューター読み取り可能なプロファイルデータセットが記憶されているデジタル記憶媒体であって、
ａ．各々のプロファイルデータセットは、作用因子が投与されている標的細胞集団から得られるサンプルに関係し、当該サンプルはＲＮＡ類及び蛋白質類の少なくとも１つを有し、
ｂ．各々のプロファイルデータセットは複数の構成要素を含み、各々の構成要素は、構成成分の測定が生物学的状態の測定を可能にするように選択された構成成分のパネル中のはっきり識別できるＲＮＡ又は蛋白質構成成分の量の定量的測定値であり、
ｃ．当該パネルは全てのプロファイルデータセットに対して同じである、
デジタル記憶媒体。
【請求項９１】
被験体からのサンプルに基づいて、被験体の生物学的状態を評価する方法であって、当該サンプルはＲＮＡ類及び蛋白質類の少なくとも１つを有し、当該方法は、
ａ．複数の構成要素を含むプロファイルデータセットであって、各々の構成要素が、構成成分の測定が生物学的状態の測定を可能にするように選択された構成成分のパネル中のはっきり識別できるＲＮＡ又は蛋白質構成成分の量の定量的測定値である、プロファイルデータセットの第１の事実をサンプルから得ること、及び
ｂ．パネルに対する較正されたプロファイルデータセットの第１の事実を作ること、ここで、較正されたプロファイルデータセットの１つの事実の各々の構成要素はプロファイルデータセットの１つの事実の対応する構成要素及びパネルに対する基準プロファイルデータセットの１つの事実の対応する構成要素の関数であり、較正されたプロファイルデータセットは被験体の生物学的状態の目安を提供すること、及び
ｃ．被験体の集団に関する複数の記録を有する状態データベースであって、各々の記録が較正されたプロファイルデータセットのはっきり識別できる１つの事実に対応する状態データベース中のデータにアクセスすること、及び
ｄ．状態データベース中のデータに対する関係において較正されたプロファイルデータセットの第１の事実を評価すること、
を含む方法。
【請求項９２】
状態データベースへのアクセスが、ネットワーク上の状態データベースにアクセスすることを含む、請求項９１の方法。
【請求項９３】
ネットワークが世界的なコンピューターネットワークである、請求項９２の方法。
【請求項９４】
較正されたプロファイルデータセットの第１の事実に関連するデータに基づいて状態データベースを補足することをさらに含む、請求項９２の方法。
【請求項９５】
生物学的状態が被験体の系に関し、当該系が、呼吸系、血管系、神経系、代謝系、泌尿系、生殖系、構造系、及び免疫系から成る群から選択され、そして構成成分のパネルが当該系に関して被験体の状態の測定を可能にする、請求項９２の方法。
【請求項９６】
各々の記録は、また、当該記録に関係して集団の特徴への参照を含み、当該特徴は、年齢グループ、性別、民族性、地理的位置、食餌、医学的不調、臨床的指標、投薬、身体活動、体重、及び環境への暴露の少なくとも１つである、請求項９２の方法。
【請求項９７】
特徴が臨床的指標である、請求項９６の方法。
【請求項９８】
生物学的状態の測定に関係する定量的遺伝子発現分析データを表示する方法であって、

ａ．複数の構成要素を含む、遺伝子発現分析データに関係する第１のプロファイルデータセットであって、各々の構成要素が、構成成分の測定が生物学的状態の測定を可能にするように選択された構成成分のパネル中のはっきり識別できるＲＮＡ又は蛋白質構成成分の量の定量的測定値である、第１のプロファイルデータセットを識別すること、
ｂ．パネルに対する較正されたプロファイルデータセットを作ること、ここで、較正されたプロファイルデータセットの各々の構成要素は第１のプロファイルデータセットの対応する構成要素及びパネルに対する基準プロファイルデータセットの対応する構成要素の関数であり、較正されたプロファイルデータセットは被験体の生物学的状態の目安を提供すること、及び
ｃ．較正されたプロファイルデータセットを図形形式で表示すること、
を含む方法。
【請求項９９】
関数が、第１のプロファイルデータセットの対応する構成要素の基準プロファイルデータセットの対応する構成要素に対する比の第２の関数である、請求項９８の方法。
【請求項１００】
関数が対数関数である、請求項９７の方法。
【請求項１０１】
図形形式が較正されたプロファイルデータセットの各々の構成要素に対する棒グラフである、請求項９７の方法。
【請求項１０２】
細胞の集団における生物学的状態の変化の記述的記録であって、
ａ．遺伝子座のパネルに関する数字で表される遺伝子発現値の第１のセットであって、当該セット中の各々の値が遺伝子座のパネル中の単一の遺伝子座に対応し、値の当該セットが第１の生物学的状態にある細胞の集団に対するプロファイルデータセットを形成する、第１のセット、
ｂ．遺伝子座のパネルに関する数字で表される遺伝子発現値の第２のセットであって、当該セット中の各々の値が単一の遺伝子座に対応し、値の当該セットが第２の生物学的状態にある第２の細胞の集団に対する基準プロファイルデータセットを形成し、値の第２のセットは、所望により、パネル中の各々の遺伝子座に対する複数の細胞の集団から得られる複数の遺伝子発現値の平均である、第２のセット、及び
ｃ．パネル中の各々の遺伝子座についての値の第１のセットの値の第２のセットに対する比に対応する数字の第３のセットであって、第３のセットは較正されたプロファイルデータセットであり、プロファイルデータセット及び較正されたプロファイルデータセットは、第２の生物学的状態に関連して第１の生物学的状態を記述する、第３のセット、
を含む、記述的記録。
【請求項１０３】
第１の細胞の集団及び第２又はそれ以上の細胞の集団が同じ細胞の集団である、請求項１０２の記録。
【請求項１０４】
第１の細胞の集団及び第２又はそれ以上の細胞の集団が異なる細胞の集団である、請求項１０２の記録。
【請求項１０５】
細胞を生物学的状態にさらすために、サンプルが被験体から得られ、当該細胞の集団が指標細胞集団である、請求項１０２の記述的記録。
【請求項１０６】
細胞の集団が被験体中にあるか又は被験体から取り出される、請求項１０２の遺伝子発現プロファイルデータセット。
【請求項１０７】
被験体の生物学的状態を診断する方法であって、
被験体からサンプルを得ること、細胞の集団をサンプルにさらすこと、及び請求項１乃至

３のいずれか１請求項に従って第２の生物学的状態に関連して第１の生物学的状態の存在を確認すること、を含む方法。
【請求項１０８】
試験化合物が予め決定された生物学的活性を有する場合、被験体が試験化合物に反応するかどうかを記述的に決定するように、被験体の生物学的状態に従って臨床試験のための被験体を選択することをさらに含む、請求項１０７の方法。
【請求項１０９】
試験化合物が製薬である、請求項１０８の方法。
【請求項１１０】
試験化合物が栄養剤である、請求項１０８の方法。
【請求項１１１】
被験体における生物学的状態に対する感受性を診断する方法であって、
ａ．被験体からサンプルを得ること、
ｂ．請求項１０２乃至１０６のいずれか１請求項に従って記述的記録を作ること、ここで、基準値のセットが、第２の生物学的状態に対する基準プロファイルデータセットのライブラリー中に含まれる第２の値の平均であり；当該ライブラリーは、予め決定された生物学的状態に従って分類された複数の基準プロファイルデータセットを含むものであること、及び
ｃ．被験体の感受性を診断すること、
を含む方法。
【請求項１１２】
生物学的状態の進行を監視する方法であって、
ａ．請求項１０２乃至１０６のいずれか１請求項に従って複数の記述的記録を作ること、ここで、第１の値の各々のセットは、その他の遺伝子発現プロファイルの各々に関して予め選択された時間間隔で決定されること、
ｂ．各々の較正されたプロファイルデータセットを較正されたプロファイルデータセットのライブラリーと比較すること、ここで、複数の較正されたプロファイルデータセットは予め決定された生物学的状態に従って分類されたものであること、及び
ｃ．生物学的状態の進行を決定すること、
を含む方法。
【請求項１１３】
作用因子に対する記述的記録を確立する方法であって、
ａ．細胞の集団を選択すること、
ｂ．細胞を作用因子にさらすこと、及び
ｃ．生物学的状態に対する標準化された基準プロファイルデータセットを使用して、請求項１０２乃至１０６のいずれか１請求項に従って記録を決定すること、
を含む方法。
【請求項１１４】
組成物が栄養物である、請求項１１３の方法。
【請求項１１５】
組成物が製薬である、請求項１１３の方法。
【請求項１１６】
組成物が感染性の作用因子である、請求項１１３の方法。
【請求項１１７】
組成物が複合混合物である、請求項１１３の方法。
【請求項１１８】
組成物の生物学的活性の確立が、組成物についての作用のメカニズムを提供することをさらに含む、請求項１１３の方法。
【請求項１１９】
組成物の生物学的活性の確立が、組成物についての代謝のメカニズムを提供することをさ

らに含む、請求項１１３の方法。
【請求項１２０】
組成物が第１の化合物及び第２の化合物をさらに含み、そして生物学的活性が、第１及び第２の化合物間の相乗作用、干渉、又は中立の相互作用に由来する、請求項１１３の方法。
【請求項１２１】
化合物が、生物学的活性が複数の化合物間の相乗作用、干渉、又は中立の相互作用に由来するような複数の化合物をさらに含む、請求項１１３の方法。
【請求項１２２】
化合物の生物学的活性が被験体に対する毒性作用である、請求項１１３の方法。
【請求項１２３】
被験体における生物学的状態を第１の生物学的状態から第２の生物学的状態へ変えるように被験体へ投与するための治療剤を治療剤の類から選択する方法であって、
ａ．被験体からのサンプルを治療剤の類の各々にさらすこと、
ｂ．請求項１０２乃至１０６のいずれか１請求項に従って各々のサンプルについての記述的記録を決定すること、
ｃ．各々の較正されたプロファイルデータセットを較正されたプロファイルデータセットのライブラリーと比較すること、ここで、較正されたプロファイルデータセットのライブラリーは予め決定された生物学的状態に従って分類されたものであること、及び
ｄ．どの治療剤が被験体における第１の生物学的状態を被験体における第２の生物学的状態に変えることができるかを決定すること、
を含む方法。
【請求項１２４】
第１の生物学的状態が、感染性作用因子、生物学的闘争作用因子、又は環境作用因子のいずれかの悪影響の結果であり、そして第２の生物学的状態がこれらの悪影響の逆転である、請求項１２２の方法。
【請求項１２５】
記述的記録のライブラリーが、単一の被験体又は単一の状態についての医学的履歴を含む、請求項１２２の方法。
【請求項１２６】
記述的記録のライブラリーが、複数の被験体又は状態についての医学的情報を含む、請求項１２２の方法。
【請求項１２７】
徴候プロファイルデータセットのライブラリーが複数の被験体からの徴候プロファイルデータセットから成る、請求項１２２の方法。
【請求項１２８】
製造プロセスによって製造された組成物の単一のバッチの生物学的有効性を特徴付けする方法であって、（ａ）請求項３の方法に従って較正されたデータプロファイルセットを提供すること；及び徴候の較正されたプロファイルデータセットを所望により含むバッチ中の各々の容器上に較正されたデータプロファイルデータセットを置くことによって組成物のバッチに標識付けすること、（ｂ）較正されたプロファイルデータセットを標準化された較正されたプロファイルデータセットと比較すること、を含む方法。
【請求項１２９】
ユーザーが利用可能な情報を作ることを含む、請求項８８のデジタル記憶媒体上の生物学的情報にアクセスする方法。
【請求項１３０】
方法が、ネットワーク、ワールド・ワイド・ウェブ、ｅメイル、インターネットアクセスサイト、又はハードコピーのいずれか上においてユーザーが利用可能な情報を作ることをさらに含む、請求項１２９の方法。
【請求項１３１】

方法が、第２のアクセスサイトへローディングするために情報にアクセスすることをさらに含む、請求項１２８の方法。
【請求項１３２】
ローディングのプロセスが情報のダウンローディングを含む、請求項１３１の方法。
【請求項１３３】
情報へのアクセスがコントロールされる、請求項１２９の方法。
【請求項１３４】
コントロールの方法がパスワードの使用を含む、請求項１３３の方法。
【請求項１３５】
ユーザーが利用可能な情報に注釈をつけることができ、当該注釈が生物学的情報の一部になる、請求項１２９の方法。
【請求項１３６】
ユーザーがデータセットに１以上の記録を追加することができ、当該１以上の記録が生物学的情報の一部になる、請求項１２９の方法。
【請求項１３７】
製品の消費者評価のための方法であって、消費者評価は徴候プロファイルに依存し、（ａ）請求項１０２に従って記述的記録を形成すること、（ｂ）記述的記録を使用して製品を識別すること、ここで、遺伝子座のパネルが徴候パネルであること、及び（ｃ）製品の説明を提供するために、較正されたプロファイルデータセットを平均の較正されたプロファイルデータセットと比較すること、を含む方法。
【請求項１３８】
製品が徴候プロファイルに従って促進される、請求項１３７の方法。
【請求項１３９】
被験体の生物学的状態を評価するため又は作用因子の使用に起因する生物学的状態を評価するためのコンピュータープログラム製品であって、コンピューター読み取り可能なプログラムコードを有するコンピューター使用可能な媒体を含み、当該コンピュータプログラムコードが、
ａ．識別可能な記録のための被験体又は作用因子からのサンプルを分類するためのプログラムコード、
ｂ．第１のデータセットを取り出すためのプログラムコードであって、第１のプロファイルデータセットが複数の構成要素を含み、各々の構成要素が、構成成分の測定が生物学的状態の測定を可能にするように選択された構成成分のパネル中のはっきり識別できるＲＮＡ又は蛋白質構成成分の量の定量的測定値であり；プロファイルデータセットが記録中に貯蔵される、プログラムコード、及び
ｃ．記録中に貯蔵するための、パネルについての較正されたプロファイルデータセットを所望により作るためのプログラムコードであって、構成されたプロファイルデータセットの各々の構成要素が第１のプロファイルデータセットの対応する構成要素及びパネルについての基準プロファイルデータセットの対応する構成要素の関数であり、較正されたプロファイルデータセットが被験体の生物学的状態の目安を提供する、プログラムコード、
を含む、コンピュータープログラム製品。
【請求項１４０】
被験体の生物学的状態を評価するため又は作用因子の使用に起因する生物学的状態を評価するためのコンピューターシステムであって、当該コンピューターシステムが、
ａ．識別可能な記録において被験体又は作用因子からのサンプルを分類するための分類モジュール、
ｂ．第１のデータセットを取り出すための取り出しモジュールであって、第１のプロファイルデータセットが複数の構成要素を含み、各々の構成要素が、構成成分の測定が生物学的状態の測定を可能にするように選択された構成成分のパネル中のはっきり識別できるＲＮＡ又は蛋白質構成成分の量の定量的測定値である、取り出しモジュール、及び
ｃ．パネルについて較正されたプロファイルデータセットを作るための作成モジュールで

あって、構成されたプロファイルデータセットの各々の構成要素が第１のプロファイルデータセットの対応する構成要素及びパネルについての基準プロファイルデータセットの対応する構成要素の関数であり、較正されたプロファイルデータセットが被験体の生物学的状態の目安を提供する、作成モジュール、を含む、コンピューターシステム。

【請求項１４１】

離れた場所で生物学的状態について患者を分析する方法であって、

ａ．生物学的状態を評価するためのプロファイルデータベースを測定するためのキットであって、遺伝子座のパネルに関してＲＮＡ又は蛋白質の定量分析のための反応体を含むキットを提供すること、

ｂ．当該パネルに対応する基準プロファイルデータセットを含む集中化されたデータベースにアクセスすること、

ｃ．患者に関して較正されたプロファイルデータセットを決定すること、及び

ｄ．較正されたプロファイルデータセットのライブラリーに関連して患者の生物学的状態を分析すること、

を含む方法。

【発明の詳細な説明】

【０００１】

技術分野

較正されたデータセットにおいて観察される変異の程度に基づいて情報を提供する遺伝子発現の変異の再生可能なパターンを識別する方法が提供される。変異は、それ自身は原因として働くことを要求されないが伝統的な種類の（ヒトに対する）臨床的指標のようなその他の非遺伝子的指標と相関している可能性がある。

【０００２】

背景的技術

医療過誤に関する議会聴聞会を含めてかなりの議論がこれまであった。医療過誤の１つの源は薬物治療についての過誤を含む。９８，０００人以上の入院患者が１年間に医療過誤の犠牲者になっていると証拠書類によって立証されている（Ｓｔａｔｅｍｅｎｔ ｏｆ ｔｈｅ Ａｍｅｒｉｃａｎ Ｐｈａｒｍａｃｅｕｔｉｃａｌ Ａｓｓｏｃｉａｔｉｏｎ ｔｏ ｔｈｅ Ｓｅｎａｔｅ Ａｐｐｒｏｐｒｉａｔｉｏｎｓ Ｃｏｍｍｉｔｔｅｅ Ｌａｂｏｒ, ｈｅａｌｔｈ ａｎｄ Ｈｕｍａｎ Ｓｅｒｖｉｃｅｓ Ｅｄｕｃａｔｉｏｎ Ｓｕｂｃｏｍｍｉｔｔｅｅ Ｈｅａｒｉｎｇ ｏｎ Ｍｅｄｉｃａｌ Ｅｒｒｏｒｓ Ｄｅｃｅｍｂｅｒ １３, １９９９）。これらの過誤は、１つより多くの薬剤を摂取する特別の患者についての薬剤の相互作用から生じる問題、特定の薬剤に対する個人の反応及び特定の状態に対する正しくない投薬に関する問題を含む。医療過誤は、さらに、診断ミスの結果として起こる。これは、鈍感な診断技術又は臨床の状態の現れ方が人によって広範囲に変化する可能性があることの結果として起こる可能性がある。現在、個人の特定の表現型及び遺伝子型を考慮に入れて医学的状態の予後の見通し、診断、及び治療を最適化するために利用可能な道具はわずかしか存在しない。

【０００３】

漢方薬又は滋養食品に対する関心が次第に大きくなってきた。これらはしばしば発展途上国において成長し、品質管理をほとんどか又は全く受けていない。滋養食品の１つのバッチは有効であるかもしれないが、第２のバッチが有効であるという保証が全くないということがよくある。さらに、滋養食品の分析は、これらの薬剤が有効成分に関してほとんど知られていない複合混合物であるため、問題が多い。

【０００４】

全ての新しい治療剤が幾つかの形態の臨床試験を必要とする。患者に対する標準的勧誘技術を使用する臨床試験において試験される腫瘍を治療するための薬剤が実際には限られた効能しか示さない可能性があることが知られている。臨床集団において観察される有益な効果が小さすぎれば、その薬剤は、一般の集団における使用に対する米政府食品医薬品局の認可を受けられない。しかしながら、観察される小さい有益な効果は、実際には、臨床

試験の計画の人為的な間違いの結果又は患者の集団における臨床的な終点であるかもしれない。薬剤の有益な効果が（もし存在するならば）検知され定量化されることを確実にするために、患者が臨床試験に入るとき患者をスクリーニングする基準を有することが望ましいだろう。

【０００５】

発明の概要

本発明の第１の態様において、被験体の生物学的状態を評価する方法であって、被験体からＲＮＡ類及び蛋白質類の少なくとも１つを有するサンプルを得ること；複数の構成要素を含む第１のプロファイルデータセットであって、各々の構成要素が、構成成分の測定が生物学的状態の測定を可能にするように選択された構成成分のパネル中のはっきり識別できるＲＮＡ又は蛋白質構成成分の量の定量的測定値である、第１のプロファイルデータセットをサンプルから得ること；及びパネルに対する較正されたプロファイルデータセットであって、当該較正されたプロファイルデータセットの各々の構成要素が第１のプロファイルデータセットの対応する構成要素及びパネルに対する基準プロファイルデータセットの対応する構成要素の関数であり、較正されたプロファイルデータセットは被験体の生物学的状態の目安を提供する、パネルに対する較正されたプロファイルデータセットを作ること、を含む方法が提供される。

【０００６】

好ましい態様において、被験体の生物学的状態を評価する方法であって、被験体から液体、細胞、及び活性作用因子の少なくとも１つを有する第１のサンプルを得ること；第１のサンプル又はその一部を指標細胞の定義された集団に加えること；指標細胞からＲＮＡ類又は蛋白質類の少なくとも１つを含む第２のサンプルを得ること；複数の構成要素を含む第１のプロファイルデータセットであって、各々の構成要素が、構成成分の測定が生物学的状態の測定を可能にするように選択された構成成分のパネル中のはっきり識別できるＲＮＡ又は蛋白質構成成分の量の定量的測定値である、第１のプロファイルデータセットを第２のサンプルから得ること；及びパネルに対する較正されたプロファイルデータセットであって、当該較正されたプロファイルデータセットの各々の構成要素は第１のプロファイルデータセットの対応する構成要素及びパネルに対する基準プロファイルデータセットの対応する構成要素の関数であり、較正されたプロファイルデータセットは被験体の生物学的状態の目安を提供する、パネルに対する較正されたプロファイルデータセットを作ること、を含む方法が提供される。

【０００７】

好ましい態様において、作用因子によって影響される生物学的状態を評価する方法であって、作用因子が投与されている細胞の標的集団から、ＲＮＡ類及び蛋白質類の少なくとも１つを有するサンプルを得ること；複数の構成要素を含む第１のプロファイルデータセットであって、各々の構成要素が、構成成分の測定が生物学的状態の測定を可能にするように選択された構成成分のパネル中のはっきり識別できるＲＮＡ又は蛋白質構成成分の量の定量的測定値である、第１のプロファイルデータセットをサンプルから得ること；及びパネルに対する較正されたプロファイルデータセットであって、当該較正されたプロファイルデータセットの各々の構成要素は第１のプロファイルデータセットの対応する構成要素及びパネルに対する基準プロファイルデータセットの対応する構成要素の関数であり、較正されたプロファイルデータセットは作用因子によって影響される生物学的状態の目安を提供する、パネルに対する較正されたプロファイルデータセットを作ること、を含む方法が提供される。

【０００８】

好ましい態様において、第２の作用因子による影響に関連して第１の作用因子による生物学的状態に対する影響を評価する方法であって、第１及び第２の作用因子がそれぞれ投与されている第１及び第２の細胞の標的集団から、ＲＮＡ類及び蛋白質類の少なくとも１つを各々が有する第１及び第２のサンプルをそれぞれ得ること；第１のサンプルから第１のプロファイルデータセットをそして第２のサンプルから第２のプロファイルデータセット

を得ること、ここで当該プロファイルデータセットの各々が複数の構成要素を含み、各々の構成要素が、構成成分の測定が生物学的状態の測定を可能にするように選択された構成成分のパネル中のはっきり識別できるＲＮＡ又は蛋白質構成成分の量の定量的測定値であるものであること；及びパネルに対する第１の較正されたプロファイルデータセット及び第２のプロファイルデータセットを作ること、ここで、（ｉ）第１の較正されたプロファイルデータセットの各々の構成要素は第１のプロファイルデータセットの対応する構成要素及びパネルに対する第１の基準プロファイルデータセットの対応する構成要素の関数であり、そして（ｉｉ）第２の較正されたプロファイルデータセットの各々の構成要素は第２のプロファイルデータセットの対応する構成要素及びパネルに対する第２の基準プロファイルデータセットの対応する構成要素の関数であり、これらの較正されたプロファイルデータセットは、第２の作用因子による影響に関連して第１の作用因子による生物学的状態への影響の目安を提供すること、を含む方法が提供される。

【０００９】

好ましい態様において、作用因子の臨床試験を行う方法であって、プラシーボ及び作用因子の選択された一方を被験体のプールの各々の候補にブラインド投与すること、及びそのような投与の影響を監視するために定量的遺伝子発現を使用すること、を含む方法が提供される。

【００１０】

好ましい態様において、コンピューター読み取り可能な較正されたプロファイルデータセットが記憶されているデジタル記憶媒体であって、較正されたプロファイルデータセットが、作用因子が投与されている標的細胞集団から得られるＲＮＡ類及び蛋白質類の少なくとも１つを有するサンプルに関し；そして較正されたプロファイルデータセットが、第１の複数の構成要素を含み、各々の構成要素が、構成成分の測定が作用因子の投与によって影響される生物学的状態の測定を可能にするように選択された構成成分のパネル中のはっきり識別できるＲＮＡ又は蛋白質構成成分の量の変化の定量的測定値である、デジタル記憶媒体が提供される。

【００１１】

好ましい態様において、被験体の集団に関する複数の記録R_iが記憶されているデジタル記憶媒体であって、各々の記録R_iは、コンピューター読み取り可能なプロファイルデータセットＰのはっきり識別できる事実P_iに対応し、ここで、プロファイルデータセットＰの各々の事実P_iは被験体から得られるはっきり識別できるサンプルに関係し、当該サンプルはＲＮＡ類及び蛋白質類の少なくとも１つを有し；プロファイルデータセットＰは複数の構成要素M_jを含み、各々の構成要素M_jは、構成成分の測定が生物学的状態の測定を可能にするように選択された構成成分のパネル中のはっきり識別できるＲＮＡ又は蛋白質構成成分の量の定量的測定値であり；各々の記録R_iは、プロファイルデータセットＰの対応するはっきり識別できる事実P_iの各々の構成要素M_{ij}に関して、構成要素M_{ij}の値に対応する値を含み；及び各々の記録R_iは、また、当該記録に関して被験体の特徴への参照を含み、当該特徴は、年齢グループ、性別、民族性、地理的位置、食餌、医学的不調、臨床的指標、投薬、身体活動、体重、及び環境への暴露の少なくとも１つである、デジタル記憶媒体が提供される。

【００１２】

好ましい態様において、多数のコンピューター読み取り可能なプロファイルデータセットが記憶されているデジタル記憶媒体であって、各々のプロファイルデータセットは、作用因子が投与されている標的細胞集団から得られるサンプルに関係し、当該サンプルはＲＮＡ類及び蛋白質類の少なくとも１つを有し；各々のプロファイルデータセットは複数の構成要素を含み、各々の構成要素は、構成成分の測定が生物学的状態の測定を可能にするように選択された構成成分のパネル中のはっきり識別できるＲＮＡ又は蛋白質構成成分の量の定量的測定値であり；及び当該パネルは全てのプロファイルデータセットに対して同じである、デジタル記憶媒体が提供される。

【００１３】

好ましい態様において、被験体からのサンプルに基づいて、被験体の生物学的状態を評価する方法であって、当該サンプルはＲＮＡ類及び蛋白質類の少なくとも１つを有し、当該方法は、複数の構成要素を含むプロファイルデータセットであって、各々の構成要素が、構成成分の測定が生物学的状態の測定を可能にするように選択された構成成分のパネル中のはっきり識別できるＲＮＡ又は蛋白質構成成分の量の定量的測定値である、プロファイルデータセットの第１の事実をサンプルから得ること；及びパネルに対する較正されたプロファイルデータセットの第１の事実を作ること、ここで、較正されたプロファイルデータセットの１つの事実の各々の構成要素はプロファイルデータセットの１つの事実の対応する構成要素及びパネルに対する基準プロファイルデータセットの１つの事実の対応する構成要素の関数であり、較正されたプロファイルデータセットは被験体の生物学的状態の目安を提供すること；及び被験体の集団に関する複数の記録を有する状態データベースであって、各々の記録が較正されたプロファイルデータセットのはっきり識別できる１つの事実に対応する状態データベース中のデータにアクセスすること；及び状態データベース中のデータに関連して較正されたプロファイルデータセットの第１の事実を評価すること、を含む方法が提供される。

【００１４】

本発明の好ましい態様において、生物学的状態の測定に関係する定量的遺伝子発現分析データを表示する方法であって、複数の構成要素を含む、遺伝子発現分析データに関係する第１のプロファイルデータセットであって、各々の構成要素が、構成成分の測定が生物学的状態の測定を可能にするように選択された構成成分のパネル中のはっきり識別できるＲＮＡ又は蛋白質構成成分の量の定量的測定値である、第１のプロファイルデータセットを識別すること、パネルに対する較正されたプロファイルデータセットを作ること、ここで、較正されたプロファイルデータセットの各々の構成要素は第１のプロファイルデータセットの対応する構成要素及びパネルに対する基準プロファイルデータセットの対応する構成要素の関数であり、較正されたプロファイルデータセットは被験体の生物学的状態の目安を提供すること、及び較正されたプロファイルデータセットを図形形式で表示すること、を含む方法が提供される。

【００１５】

好ましい態様は、細胞の集団における生物学的状態の変化の記述的記録であって、遺伝子座のパネルに関する数字で表される遺伝子発現値の第１のセットであって、当該セット中の各々の値が遺伝子座のパネル中の単一の遺伝子座に対応し、値の当該セットが第１の生物学的状態にある細胞の集団に対するプロファイルデータセットを形成する、第１のセット、遺伝子座のパネルに関する数字で表される遺伝子発現値の第２のセットであって、当該セット中の各々の値が単一の遺伝子座に対応し、値の当該セットが第２の生物学的状態にある第２の細胞の集団に対する基準プロファイルデータセットを形成し、値の第２のセットは、所望により、パネル中の各々の遺伝子座に対する複数の細胞の集団から得られる複数の遺伝子発現値の平均である、第２のセット、及びパネル中の各々の遺伝子座についての値の第１のセットの値の第２のセットに対する比に対応する数字の第３のセットであって、第３のセットは較正されたプロファイルデータセットであり、プロファイルデータセット及び較正されたプロファイルデータセットは、第２の生物学的状態に関連して第１の生物学的状態を記述する、第３のセット、を含む、記述的記録に関する。

【００１６】

好ましい態様において、被験体の生物学的状態を診断する方法であって、被験体からサンプルを得ること、細胞の集団をサンプルにさらすこと、及び請求項１乃至３のいずれか１請求項に従って第２の生物学的状態に関連して第１の生物学的状態の存在を確認すること、を含む方法が提供される。

【００１７】

好ましい態様において、被験体における生物学的状態に対する感受性を診断する方法であって、被験体からサンプルを得ること、上記に従って記述的記録を作ること、ここで、数値の基準セットが、第２の生物学的状態に対する記述的記録のライブラリー中に含まれる

第２の値の平均であり；当該ライブラリーは、予め決定された生物学的状態に従って分類された複数の基準プロファイルデータセットを含むものであること、及び被験体の感受性を診断すること、を含む方法が提供される。

【００１８】

好ましい態様において、生物学的状態の進行をモニターする方法であって、上記に従って複数の記述的記録を作ること、ここで、第１の値の各々のセットは、その他の遺伝子発現プロファイルの各々に関して予め選択された時間間隔で決定されること、各々の較正されたプロファイルデータセットをプロファイルデータセットのライブラリーと比較すること（ここで較正されたプロファイルデータセットは予め決定された生物学的状態に従って分類されている）、及び生物学的状態の進行を決定すること、を含む方法が提供される。

【００１９】

好ましい態様において、組成物の生物学的活性を確立する方法であって、細胞の集団を選択すること、細胞を組成物にさらすこと、及び生物学的状態に対する標準化された基準プロファイルデータセットを使用して、上の記述に従って記録を決定すること、を含む方法が提供される。

【００２０】

好ましい態様において、被験体における生物学的状態を第１の生物学的状態から第２の生物学的状態へ変えるように被験体へ投与するための治療剤を治療剤の類から選択する方法であって、被験体からのサンプルを治療剤の類の各々にさらすこと、上記のいずれか１の方法に従って各々のサンプルについての記述的記録を決定すること、各々の較正されたプロライルデータセットを較正されたプロファイルデータセットのライブラリーと比較すること、ここで、較正されたプロファイルデータセットのライブラリーは予め決定された生物学的状態に従って分類されたものであること、及びどの治療剤が被験体における第１の生物学的状態を被験体における第２の生物学的状態に変えることができるかを決定すること、を含む方法が提供される。

【００２１】

好ましい態様において、製造プロセスによって製造された組成物の単一のバッチの生物学的有効性を特徴付けする方法であって、上の方法のいずれか１により、フィンガープリント（ｆｉｎｇｅｒｐｒｉｎｔ）又は徴候プロファイルを提供すること、バッチ中の各々の容器上にフィンガープリント（徴候プロファイル）を置くことによって組成物のバッチに標識付けすること、を含む方法が提供される。

【００２２】

好ましい態様において、製品の消費者評価のための方法であって、消費者評価は徴候プロファイルに依存し、徴候プロファイルを使用して製品を識別すること、を含む方法が提供される。

【００２３】

好ましい態様において、被験体の生物学的状態を評価するため又は作用因子の使用に起因する生物学的状態を評価するためのコンピュータープログラム製品であって、コンピューター読み取り可能なプログラムコードを有するコンピューター使用可能な媒体を含み、当該コンピュータプログラムコードが、識別可能な記録のための被験体又は作用因子からのサンプルを分類するためのプログラムコード、第１のデータセットを取り出すためのプログラムコードであって、第１のプロファイルデータセットが複数の構成要素を含み、各々の構成要素が、構成成分の測定が生物学的状態の測定を可能にするように選択された構成成分のパネル中のはっきり識別できるＲＮＡ又は蛋白質構成成分の量の定量的測定値であり；プロファイルデータセットが記録中に貯蔵される、プログラムコード、及び記録中に貯蔵するための、パネルについての較正されたプロファイルデータセットを所望により作るためのプログラムコードであって、構成されたプロファイルデータセットの各々の構成要素が第１のプロファイルデータセットの対応する構成要素及びパネルについての基準プロファイルデータセットの対応する構成要素の関数であり、較正されたプロファイルデータセットが被験体の生物学的状態の目安を提供する、プログラムコード、を含む、コンピ

ュータープログラム製品が提供される。
【００２４】
本発明の好ましい態様において、被験体の生物学的状態を評価するため又は作用因子の使用に起因する生物学的状態を評価するためのコンピューターシステムであって、当該コンピューターシステムが、識別可能な記録において被験体又は作用因子からのサンプルを分類するための分類モジュール、第１のデータセットを取り出すための取り出しモジュールであって、第１のプロファイルデータセットが複数の構成要素を含み、各々の構成要素が、構成成分の測定が生物学的状態の測定を可能にするように選択された構成成分のパネル中のはっきり識別できるＲＮＡ又は蛋白質構成成分の量の定量的測定値である、取り出しモジュール、及びパネルについて較正されたプロファイルデータセットを作るための作成モジュールであって、構成されたプロファイルデータセットの各々の構成要素が第１のプロファイルデータセットの対応する構成要素及びパネルについての基準プロファイルデータセットの対応する構成要素の関数であり、較正されたプロファイルデータセットが被験体の生物学的状態の目安を提供する、作成モジュール、を含む、コンピューターシステムが提供される。
【００２５】
好ましい態様において、離れた場所で生物学的状態について患者を分析する方法であって、生物学的状態を評価するためのプロファイルデータベースを測定するためのキットであって、遺伝子座のパネルに関してＲＮＡ又は蛋白質の定量分析のための反応体を含むキットを提供すること、当該パネルに対応する基準プロファイルデータセットを含む集中化されたデータベースにアクセスすること、患者に関して較正されたプロファイルデータセットを決定すること、及び較正されたプロファイルデータセットのライブラリーに関連して患者の生物学的状態を分析すること、を含む方法が提供される。
【００２６】
本発明の好ましい態様は、第２のリモート位置から採取された試料から、被験体の１つの部位での生物学的状態を決定するための較正されたプロファイルデータベースの使用を含む。生物学的状態は、疾病、治療介入、老化、健康調整、運動、毒素にさらすこと、感染の状況及び健康の状況を含んでもよい。例えば、較正された正確なプロファイルは、同じ被験体から細胞のサンプリングにより１つの部位（例えば、肝臓）での生物学的状態（類）を測定するのに用いられ得るが、異なる部位では生物学的状態に対する標的とは一般に考えられない（例えば、肝臓病の場合での、末梢血液細胞）。
【００２７】
本発明の好ましい態様は、生物学的状態を評価するために指標細胞に細胞又は液体試料を置くことを含む被験体の生物学的状態を決定するための較正されたプロファイルデータベースを含み、生物学的状態は、疾病、治療介入、老化、健康調整、運動、毒素にさらすこと、感染の状況及び健康の状況を含む。
【００２８】
本発明の好ましい態様は、未知の性質の２つの作用因子の比較、即ち単純な混合物である作用因子に対する複合混合物である作用因子の比較、及び作用因子の部類に対する単一の作用因子の比較、を含む、治療剤及び治療剤候補の生物活性を評価、比較及び対比するために較正されたプロファイルデータベース及びプロファイルの使用を含む。
【００２９】
本発明の好ましい態様は、生体内での活性（活性は効能と毒性を含み）を予測するため、指標細胞、又は生体外の液体又は細胞中に試験管内での作用因子の投与から得られる較正されたプロファイルデータベースの使用を含み、明細書で記載されるように長期の活性を予測するために、生体内で短期の作用因子の投与についてのデータを更に許容する。
【００３０】
本発明の好ましい態様は、少なくとも１つのデータベース及びその使用で、該データベースは、疾病、地理的位置、民族、年齢、及び健康状態を含む因子に従い認定される識別できる集団についての較正されたプロファイルデータセットと基準プロファイルデータセッ

トの少なくとも１つを含む。
【００３１】
本発明の好ましい態様は、時間にわたる個体に対応するデータベース、個人向けの健康医療プログラムを管理することを含むその使用である。
【００３２】
更なる態様は、細胞集団への作用因子の効果についての試験管内及び生体内での研究からの較正されたプロファイルデータを含む較正されたプロファイルデータ及びデータベースを用いる臨床試験を実施する方法、及び較正されたプロファイルデータ及び従来の医学的データを用いる臨床研究ネットワークを作る方法を含む。
【００３３】
特定の態様の詳細な説明
この説明及び添付の請求の範囲で使用されているように、以下の語句は、文脈が要求しない限り、指示された意味を持つ。
【００３４】
「細胞のコレクション（ｃｏｌｌｅｃｔｉｏｎ）」は、細胞のセットであり、該組は少なくとも１つの構成成分を有する。
【００３５】
「細胞の集団（ｐｏｐｕｌａｔｉｏｎ）」は、１以上の細胞を含む。細胞の集団は、生体内の細胞又は試験管内の培養に当てはまり得る。試験管内の培養は器官培養又は細胞培養を含み得、細胞培養は、真核又は原核細胞からなる一次又は連続細胞培養であり得る。細胞ラインは、例えば、腫瘍から、血液又は血液の一部からの一次培養又は細胞の試料、又は器官からのバイオプシー外植片（ｂｉｏｐｓｙ ｅｘｐｌａｎｔ）であってもよく、又は定着した細胞ライン又は微生物株であってもよい。
【００３６】
蛋白質が得られる「被験体の部位」は、細胞の採集又は細胞の集団が得られた同じ被験体の部分であり得る（必ずしもその必要はない）。例えば、細胞と蛋白質の両方が被験体の血液から得られてもよい。また、例えば、細胞が血液から得られ、蛋白質が組織のこすり（ｓｃｒａｐｉｎｇ）又は逆から得られてもよい。同様に、蛋白質は例えば尿から得られてよく、一方細胞は例えば血液からのようにどこからでも得られてもよい。
【００３７】
遺伝子の「パネル（ｐａｎｅｌ）」は、少なくとも２つの構成構成成分を含む遺伝子の組である。
【００３８】
組成物が投与される被験体の「標準の」状態は、被験体がたまたま病気で苦しんでいる場合であっても、投与前の被験体の状態を意味する。
【００３９】
遺伝子の「発現（ｅｘｐｒｅｓｓｉｏｎ）」は、メッセンジャーＲＮＡの翻訳から生じるメッセンジャーＲＮＡ又は蛋白質いずれかの遺伝子生成物を含む。
【００４０】
遺伝子の共通のパネルに基づいた「多数」のデータセットは、同じパネルに基づいたデータセットの例に関して、統計的に重要な結論が導かれることを許容する十分に大きなデータセットの数である。
【００４１】
被験体の「生物学的状態」は、観察下にある適切な範囲の被験体の状態であり、そのような範囲は、健康、癌、外傷、老化、感染、組織再生、発育段階、身体活動、肥満を含む疾病、又は心的状態のような状態の変化のモニターされ得る被験体の任意の側面を含み得る。理解されるように、状態は慢性又は急性又は単に一時的であり得る。更に、目標とした生物学的状態は、細胞の生体又は集団を通じてはっきり表れ得、又は特定の器官（皮膚、心臓、目、又は血液のような）に限定され得る。「生物学的状態」の語は、「心理学的状態」を含む。

【００４２】
臨床試験での被験体への組成物の選択された１つ又はプラシボー（ｐｌａｅｃｂｏ）の「盲検投与」は、被験体が投与された物質が組成物又はプラシボーであるかの知識が不足している臨床試験計画表に応じて被験体に組成物又は擬薬を投与することを含む。
【００４３】
「生物（ｏｒｇａｎｉｓｍ）」は、微生物、動物、及び植物を含む任意の生きている細胞を含む。この文脈中では、動物は一般に哺乳類であるが、ゼブラダニオ（ｚｅｂｒａ ｆｉｓｈ）のような脊椎動物の非哺乳類や、線虫（Ｃａｅｎｏｒｈａｂｄｉｔｉｓ ｅｌｅｇａｎｓ）のような無脊椎動物であり得る。
【００４４】
「作用因子（ａｇｅｎｔ）」は、組成物又は刺激（ｓｔｉｍｕｌｕｓ）である。「刺激」は、例えば、紫外線Ａ又はＢ、季節感情疾患用の光治療、又はソラレンでの乾癬の治療、又は埋め込まれた放射性種、他の放射性照射での黒色腫の治療を含み得る。「組成物」は、化学的化合物、栄養物、化合物の組合せ、又は複合混合物を含む。
【００４５】
「臨床的指標（ｃｌｉｎｉｃａｌ ｉｎｄｉｃａｔｏｒ）」は、細胞の集団又は生物の生理学的条件を評価する際に、単独で又は他のデータと結合して用いられる任意の生理学的データである。
【００４６】
「徴候パネル（ｓｉｇｎａｔｕｒｅ ｐａｎｅｌ）」は、構成成分の下位分類を表すパネルで、構成成分の下位分類は、データセットの各々の構成要素で与えられた生物学的条件に関わる情報の相対的高い段階に応じて選択される。
【００４７】
構成成分のパネル中の「はっきり識別できる（ｄｉｓｔｉｎｃｔ）ＲＮＡ又は蛋白質構成成分」は、ＲＮＡ及び蛋白質の少なくとも１つを含むパネルであり、パネルの各々の構成構成成分は独特である。
【００４８】
本発明の好ましい態様は、生物学的条件又は生物学的条件への作用因子の効果を記述する較正されたデータセットの構成である。較正されたデータセットは、遺伝子発現での変化（この変化は有益である）に対応する数値のセットを表現する。このアプローチは、特定の状態に関係する標的細胞の全ての遺伝子発現の包括的な解析を必要としない。特定の重要ないずれか１つの遺伝子発現も必要ではない。むしろ、再現性のある方法で特定の状態と関係づける変化のパターン（プロファイル）が求められる。相関について先行知識はないであろうが、相関は合理的な大きさの構成成分からなるパネル（例えば１００までの構成成分）を評価し、異なる被験体について、又は特定の状態に最も有益な座が選択され得る同じ被験体についての遺伝子発現プロファイルを反復的に試験することにより確立され得る。特定の状態に対して一般して変化するパネル中構成構成成分の有益な下位構成要素が選択され得、それからこの下位構成要素は徴候パネルとなり、該徴候パネルは徴候プロファイル（ｓｉｇｎａｔｕｒｅ ｐｒｏｆｉｌｅ）となる。
【００４９】
本発明の更なる態様において、単一の徴候パネルを反映しているより多くの構成要素を有する個体についての任意の較正されたデータセットは、遺伝子セットへの生物学的状態の作用機構について新しい洞察を潜在的に提供する追加の徴候パネルに対応する較正されたプロファイルに利用され得る。環境の変化又は老化の結果、細胞中の転写されたＲＮＡの変化の測定は、細胞の応答のこの上なく敏感な測定である。細胞中の転写されたＲＮＡを定量化するのに今日使える技術は、このアプローチの感度を増す。本発明の好ましい態様、これは転写されたＲＮＡ量の変化のパターンに関連するが、この豊富な情報に焦点を合わせ、解釈する方法を提供する。
【００５０】
上のアプローチと対照的に、先行文献において多くの注意が、ヒトゲノムのシーケンスと

その中にコード化された全ての遺伝子の識別に向けられてきた。シーケンスデータ量が大きくなるに伴い、微小配列（ｍｉｃｒｏａｒｒａｙ）が突然変異についての数千の遺伝子シーケンスを調査する方法を提供する。微細配列は、個体中の突然変異を識別するＤＮＡプロファイルを提供するのに用いられてきており、これらの突然変異は、これらの個体中の疾病の進展に関する予想に関係するであろう。トランスクリプトミックス（ｔｒａｎｓｃｒｉｐｔｏｍｉｃｓ）とプロテオミックス（ｐｒｏｔｅｏｍｉｃｓ）は現在ますます増加する注意の焦点である。これらの研究は、生体細胞で生産されるＲＮＡと蛋白質の全体を解析することに向かっている。微小配列は、発現されるかそしてどの細胞によって発現されるかについて、何千という異なるヒトＲＮＡを解析する方法を提供する。例えば、様々な型の癌細胞により生産されるｍＲＮＡ類を調査するために国立癌研究所とその他で行われているプロジェクトは、１以上の癌に活性な５０、０００の遺伝子を明らかにした。これらの研究の目標は、特定の蛋白質の生産を不能にするか又は高めることに向けられる新規な癌の薬剤を識別することである（Ｋａｔｈｒｙｎ ＢｒｏｗｎのＴｈｅ Ｈｕｍａｎ Ｇｅｎｏｍｅ Ｂｕｓｉｎｅｓｓ Ｔｏｄａｙ、Ｓｃｉｅｎｔｉｆｉｃ Ａｍｅｒｉｃａｎ、２０００年7月、５０頁；Ｊｕｌｉａ ＫａｒｏｗのＴｈｅ“Ｏｔｈｅｒ”Ｇｅｎｏｍｅｓ、Ｓｃｉｅｎｔｉｆｉｃ Ａｍｅｒｉｃａｎ、２０００年7月、５３頁；Ｋｅｎ Ｈｏｗａｒｄ、“Ｔｈｅ Ｂｉｏｉｎｆｏｒｍａｔｉｃｓ Ｇｏｌｄ Ｒｕｓｈ、Ｓｃｉｅｎｔｉｆｉｃ Ａｍｅｒｉｃａｎ、２０００年7月、５８頁；Ｃａｚｏｌ Ｅｚｚｅｌｌ、Ｂｅｙｏｎｄ ｔｈｅ Ｈｕｍａｎ Ｇｅｎｏｍｅ、Ｓｃｉｅｎｔｉｆｉｃ Ａｍｅｒｉｃａｎ、２０００年7月、６４頁；これら全ては参照により取込まれている）。個体類の遺伝的変化と、健康と病気における遺伝子の機能的な内部関係を関係づけるのに大きな努力が、単一の核酸多形性の協会及びヒトエピゲノム（Ｅｐｉｇｅｎｏｍｅ）協会（Ｂｅｃｋ等、Ｎａｔｕｒｅ Ｂｉｏ Ｔｅｃｈｎｏｌｏｇｙ １７（１９９９年）１１４４頁）を含む様々な協会で行われてきた。エピゲノム協会は、５００の異なるヒト組織中の健康な及び病気の個体両方からゲノム断片のセットを解析することを計画している（Ｂｉｏｗｏｒｌｄ Ｉｎｔｅｒｎａｔｉｏｎａｌ：１９９９年１２月２２日）。このアプローチは、特定の状態に関係する絶対的な遺伝子発現とその状態の存在とを関係づけることを求める。減色法による、又はハウスキーピング遺伝子に関する量の決定による、又は１つの遺伝子発現システムを標的化による、を含む絶対量で遺伝子発現を測定することを求める先行技術の例は、米国特許第５、６４３、７６５号、米国特許第５、８１１、２３１号、米国特許第５、８４６、７２０号、米国特許第５、８６６、３３０号、米国特許第５、９６８、７８４号、米国特許第５、９９４、０７６号、国際公開公報９７／４１２６１、国際公開公報９８／２４９３５、国際公開公報９９／１１８２２、国際公開公報９９／４４０６３、国際公開公報９９／４６４０３、国際公開公報９９／５７１３０、国際公開公報００／２２１７２、及び国際公開公報００／１１２０８である。

【００５１】

本発明者等は、試料と基準、例えば該状態のある被験体とその状態が欠けている被験体、間での変化の程度により有益で、遺伝子発現の変化の再現性あるパターンを識別することにより上記に対して異なる新規なアプローチを取った。該変化は、伝統的な性質の臨床的指標（ヒトに対する）のような他の非遺伝的な表示と関連づけられ得るが、必ずしも本質的に原因とならない。従って、特定の環境下で細胞中の遺伝子座により生産される遺伝子発現性生物（例えばＲＮＡ転写）の量が測定され、それから第１のプロファイルデータセット中の値として蓄えられる。この値は第２の値（基準プロファイルデータセット）に関して較正された、較正されたプロファイルデータセットの構成要素を与える。較正されたデータセットを生み出す特定の基準データセットをあてにするプロファイルデータセットに記録された値は、記述的記録の部分となり、何れかの世界の場所で測定されたプロファイルデータ又は較正されたプロファイルデータセットの形の任意の新しいデータが直接、較正されたプロファイルデータセット及び基準データセットを含む記述的記録の記録文書と直接比較されることができ、その結果蓄えられたプロファイルのライブラリーを広げ、そして特定の生物学的条件又は作用因子についての予測的又は診断上のデータを提供する

ように、その記録のいずれか又は全ては世界的ネットワークを通じてアクセスできるデータベースに蓄えられ得る。

【００５２】

本発明者等は、遺伝子座に対応する構成成分からなる選択されたパネル（そこからの定量的な遺伝子発現が、例えば被験体の試料中の転写されたＲＮＡを定量的に測定することによる目安である）を、次を含む適用について使用することを例証する。即ち、（ａ）標的とされる生理学的状態の範囲に対して、個々に調合され得る天然組成物又は合成組成物又は刺激、組合せ又は混合物からなる療法の効能の測定、（ｂ）個体又は集団に対する組成物又は組成物の混合物の毒物学的効果及び投与の有効性、（ｃ）毒物活性の相乗作用、付加性、陰性、中性を検出するため、１回の治療で投与された２種の異なる作用因子がどのように相互作用するかの決定、（ｄ）病気の状態を明らかにするために有効なプロファイルデータセットにより被験体を予め選択する新しい基準を提供することにより前臨床及び臨床試験を行い、フェーズ１又は２の試験を行う前に、これら被験体に対し予備的な投与調査を行うこと。遺伝子発現プロファイリングが、フェーズ３臨床試験のコストを低減するために用いられ得、フェーズ３より先の試験でも用いられ得る、（ｅ）認可された薬のラベル化、（ｆ）独特な生理に向けられている特定の被験体用の薬物構成要素からの適切な薬物の選択、（ｇ）症状の徴候に先立つ医学的状態又は感染の予兆の診断又は決定、あるいは治療剤の投与に関係する副作用の診断、（ｈ）被験体の健康管理を行うこと、及び（ｉ）１種の作用因子又は作用因子の混合物の異なるバッチに対する品質管理、である。

【００５３】

被験体

ここでの方法は、任意の生きている生物を含む被験体に適用され得、生きている生物は、バクテリアのような原核生物、又はスペクトルの一端での単一細胞の真核生物、及びもう一方端でのヒト、及び植物を含むその間の全てを含む真核生物を含む。図は、ヒト及び哺乳類から得られる較正されたプロファイルデータセットに関係する。それにも関わらず、ここで開示された方法は、当業者による過度な説明の必要なく他の生物の細胞に適用され得る。これは、全ての細胞がＲＮＡを転写し、どのように全ての型の細胞からＲＮＡを抽出するかは本技術分野では公知だからである。

【００５４】

組織の試料は、単細胞又は多細胞又は細胞の断片を含み得る。体液は、被験体の血液、尿、脊髄液、リンパ、粘膜分泌物、血液リンパ、又はその他公知の体液を含む。動物の被験体に対して、組織又は流体の試料は、バイオプシー（ｂｉｏｐｓｙ）の注射針吸引、洗浄試料、けずり取り及び外科的切開の方法、又はその他公知の方法により得ることができる。

【００５５】

パネル

パネル中の構成成分の選択の工程は、特定の生物学的状態で直接又は間接に変化するＲＮＡ又は蛋白質、又はＲＮＡ類又は蛋白質類のセットに関する公的に入手できる医学文献を調査することを含む。１００までの構成成分を含むパネルが選択され得る。調査される条件により、パネル構成成分のほんの小さな部分集合が有益であり得る。遺伝子のパネルの会員を決める際に、パネルが網羅的選択である必要はない。むしろ、該パネルから、目標の生理学的又は生物学的状態について一貫して識別する遺伝子発現プロファイルを得ることが望ましい。更に、パネルは、生物学的効果に直接応答する細胞中の遺伝子発現の期待されるプロファイルに応じて必ずしも選択されない。例えば、肝臓の物質代謝に関連する遺伝子発現は、血液の試料中で解析され得る。図２０及び２２は、肝臓の物質代謝のマーカーを用いるハーブ（薬用植物）の作用因子で処理された全血の較正されたプロファイルを提供する。

【００５６】

パネル中の構成成分の数は変わり得る。以下で与えられる例によると、２４までの遺伝子からなるパネルが遺伝子発現レベルを評価するために選択される。パネルは１００程度の

構成成分であり得るが、特定のパネルがわずか２４の構成成分を有し、とりわけ１２未満の構成成分を有することが望ましい。例えば、より大きなパネルから得られるわずか８つの遺伝子からなる部分集合が用いられてきており、これは識別を成し遂げるのに十分有益である。遺伝子発現がモニターされるパネル中の構成成分の数は、文脈に応じて広く変わり得る。例えば、図１は、試験管中の細胞培養から及び動物毒物性試験から得られたデータを記載し、これは約２５乃至１００又はそれ以上の遺伝子の遺伝子発現を含む。対照的に、マーカー又は代理のマーカーの選択は、臨床試験で得られる試料から分析される、例えば3乃至１００の遺伝子、好ましくは5乃至５０又は5乃至２５の遺伝子を含む。このように、遺伝的疾病素因、療法剤への応答、炎症状態、又は感染等のような医学的状態についての予測値を有するマーカー又は代理マーカーが識別され得、相関を改良するため累積的に大きな集団を得ることができる。それから、小容量の血液試料を用いて、健康プロファイルが個々の被験体に対して記述される。血液試料は、多くの医学的状態をのマーカー又は代理マーカーを含む、約１００乃至５００の遺伝子の遺伝子発現プロファイルデータについて分析され得る（図１：右のパネル）。様々なサイズのパネルが利用され得る方法論における必要で後に続く改良が、１５の遺伝子又は１２の遺伝子と同じ大きさのパネル又は、６、５、４、３、又は2の遺伝子と同じ小ささのパネルを有する部分集合の選択に導き得る。

【００５７】

いずれか1つの生物学的条件が、徴候較正されたプロファイル（フィンガープリント（ｆｉｎｇｅｒｐｒｉｎｔ）とも呼ばれる）を提供する少量の非常に有益な構成成分を有する徴候パネルにより記述され得ると予想される。非常に有益な位置の存在は、いくつかの貼付図面において示される。例えば、図１１（ａ）Ｉ１－２、Ｉ１－４、及びＩ１－５は非常に有益のように見える。図２１中の非常に有益な構成成分はインターロイキンを含む。徴候パネルは、特定の生物学的状態又は生物学的状態への特定の作用因子の効果を記述する際に標準として働くのに十分強固な折プロファイル又は指紋を提供し得る。

【００５８】

徴候パネルを例示する目的で、特定の生物学的状態について有益な、炎症を測定するためのパネルの構成成分が提供された。例えば、薬剤の様々な濃度への５人の被験体の応答を決めるために、図１８（ａ）－（ｅ）で、6つの構成成分（Ｉ１－ａ、Ｉ１－６、Ｉ１－８、Ｉ１－１８、ＧＭＣＳＦ及びＩＮＦ－ｇ）を有する炎症に対するパネルを用いた。この構成成分の類は、図１９ａ及び図１９ｂで示されたように遺伝子の位置に関連するより大きな炎症のパネルからなる部分集合であり、炎症パネルは、Ｉ１－ａ、Ｉ１－ｂ、Ｉ１－２、Ｉ１－３、Ｉ１－４、Ｉ１－６、Ｉ１－７、Ｉ１－８、Ｉ１－１０、Ｉ１－１２ｐ４０、Ｉ１－１５、Ｉ１－１５、Ｉ１－１８、ＧＭ－ＣＳＦ、Ｉｆｎ－ガンマ、ＴＧＦ－ｂ、ｃｏｘ－２、ＩＣＥ、ＭＭＰ－９、ＩＣＡＭ、ＴＮＦ－ａ、及びＴＮＦ－ｂを含む。構成成分の部分集合は、生物学的状態に関して要求される情報を基準に選択された。

【００５９】

本発明の態様は、別々に又は一緒に使用され得る少なくとも４つのパネルの例を提供する。これらのパネルは、炎症パネル（ＴＮＦ－ａ、Ｉ１－ｂ、ＩＣＡＭ、Ｉ１－８、Ｉ１－１０、Ｉ１－１２ｐ４０、ＩＣＥ、ｃｏｘ－２、ｃｏｘ－１、及びｍｍｐ－３）、細胞成長及び分化パネル（ｃ－ｆｏｓ、ｃ－ｊｕｎ、及びＳＴＡＴ３）、毒性パネル（ＳＯＤ－１、ＴＡＣＥ、ＧＲ、ＨＳＰ７０、ＧＳＴ、ｃ－ｆｏｓ、ｃ－ｊｕｎ、ＩＮＯＳ）及び肝臓の物質代謝パネル（ＩＮＯＳ、ｃｙｐ－ａ、及びｕ－ｐａ）である。他のパネルは、皮膚応答又は前立腺癌又は内皮／心臓血管の応答パネル又は細胞成長又は分化又は肝臓の物質代謝パネルを含む。例として提供されるが、上のパネルはこれらに制限することを意図していない。

【００６０】

遺伝子発現

試料中の特定のＲＮＡ量を測定するため、本発明者等は、パネルの構成成分に対し試料から転写されたＲＮＡを抽出し定量化する当業者に知られた方法を用いた。ＲＮＡは、組織

、体液、又は被験体の集団が成長し得る培地媒体のような試料から抽出される。例えば、細胞は崩壊し、ＲＮＡはＤＮアーゼ反応を行うための適用な溶液内に溶出し得る。最初の鎖合成は逆転写酵素を用いて行われ得る。遺伝子増幅、とりわけ定量ＰＣＲアッセイが行われ、興味あるサイズの遺伝子が１８５ｒＲＮＡのようなマーカーに対し較正される（Ｈｉｒａｙａｍａ等、Ｂｌｏｏｄ ９２、１９９８年、４６－５２頁）。試料は、複数通り（例えば、４回反復）で測定される。ｍＲＮＡの相対量は、サイズマーカーと興味ある遺伝子間のしきいサイクルの相違で決定される。本発明の態様によると、定量ＰＣＲは、増幅を用いて、ＰＥ Ｂｉｏｓｙｓｔｅｍｓ（フォスター市、カリフォルニア州）により商業的に支給されるような作用因子及び機器を報告して行われる。標的転写の増幅の明確な効率が与えられ、増幅された標的テンプレートからのシグナルが検知できる点（例えば、サイクル数）が、測定された試料中の特定のメッセージ転写の量に直接関係づけられ得る。同様に、蛍光、酵素活性、１分間あたりの分解、吸収等のような、量で表される他のシグナルは、標的テンプレートの既知の濃度に関係づけられ（例えば、引用標準曲線）又は限定された変異性で標準に規格化された時に、未知試料中の標的テンプレートの数を定量化するのに用いられ得る。

【００６１】

増幅方法に限定されないが、定量的遺伝子発現技術は標的転写の増幅を利用し得る。代わりに又は標的転写の増幅と組合わせて、レポーターシグナルの増幅も用いられ得る。標的テンプレートの増幅は、等温遺伝子増幅戦略又はＰＣＲのような熱サイクルによる遺伝子増幅により達成され得る。増幅された標的又はレポーターと開始テンプレートの濃度の説明でき再生できる関係うぃ得ることが望ましい。

【００６２】

例えばミクロな液体及び高感度なマーカーを用いる当該技術分野の技術は、ＲＮＡの定量化が単細胞又は崩壊した細胞から直接起こることを可能にするであろう。これはマーカーの増幅に依存するが、転写の増幅自体は必要としないであろう。いずれか特定の位置に対し測定される転写の量は、特定のパネルに対する第１のプロファイルデータセットからなるデータ点又は構成要素である。

【００６３】

本発明の態様によると、第１のプロファイルデータセットは試料から得られ、第１のプロファイルデータセットは複数の構成要素を含み、各々の構成要素は遺伝子座から転写されるＲＮＡ量の定量的目安であり、遺伝子座は構成要素からなるパネル中の構成要素である。第１のプロファイルデータセットは、遺伝子座に対応する識別できるＲＮＡ又は蛋白質の量の定量的目安から得られ得る。ここで与えられる図は、ＲＮＡに向けられている。しかし、該方法は蛋白質を用いて実施することもでき、感度のある定量的技術が細胞中の識別できる蛋白質の量を測定するのに利用できる。

【００６４】

基準プロファイルデータセット

単一の個体から及び個体類の大きな群からの試料の分析は、特定のパネル又は一連のパネルに関するプロファイルデータセットのライブラリーを提供する。これらのプロファイルデータセットは、基準プロファイルデータセットとしての用途にライブラリー中に記録として蓄えられる。「基準（ｂａｓｅｌｉｎｅ）」の語が示唆しているように、蓄えられた基準プロファイルデータセットは、生物学的条件又は作用因子について有益である較正されたプロファイルデータセットを提供するコンパレーターとして働く。多くの基準プロファイルデータセットがライブラリーに蓄えられ、多くの相互参照の仕方で分類されるだろう。分類の１つの型は、データセットが得られるパネルの特性に依存するかもしれない。分類のもう１つの型は特定の生物学的条件の使用であるかもしれない。生物学的条件の概念は、細胞又は細胞集団が任意のある時間にある任意の状態を包含する。この状態は、試料の地理的位置、被験体の性別、又はその他任意の識別器を反映する。識別器のいくつかは重なり得る。ライブラリーは、１つの被験体又は特定の臨床試験に関する記録についてアクセスもされ得る。基準プロファイルデータセットの分類は、特定の被験体、医学的状

態、特定の作用因子についての医学的情報で更に注釈づけられ得る。

【００６５】

較正されたプロファイルデータセットを形成するための基準プロファイルデータセットの選択は、評価、モニター、又は予測される生物学的条件、並びに較正されたパネルの意図した用途（例えば、薬剤の開発、品質管理、又は他の用途をモニターする）に関連する。第１のプロファイルデータセットが得られる同じ被験体から、又は様々な回数での、刺激、薬剤又は複合化合物にさらされた異なる被験体から、又は同じか異なる集団から得られ得る基準プロファイルデータセットにアクセルすることが望ましいかもしれない。

【００６６】

プロファイルデータセットは、第１のデータセットが得られる同じ被験体から生じ、試料は別の又は類似の時間、異なる又は類似の部位、又は異なるか類似の生理学的条件で採取される。図５は、試料が刺激の前又は刺激の後に採取される臨床試験計画書を提供する。刺激されていない試料から得られたプロファイルデータセットは、刺激後に採取された試料に対する基準プロファイルデータセットとして働き得る。基準データセットは、いくつかの明らかに特有な又は生物学的状態を有する被験体の集団のプロファイルデータを含むライブラリーからも得られ得る。そして、結果として得られる較正されたプロファイルデータセットは、基準プロファイルデータベース及び所望により第１のプロファイルデータセット（第１のプロファイルデータセットは、適当な分類基準の下では通常基準プロファイルデータセットに取り込まれるであろうが）に沿って又は基準プロファイルデータベースから離れて、データベース又はライブラリー（図６）中の記録として蓄えられ得る。

【００６７】

選択された基準プロファイルデータセットは、効能、毒性等の点から製造ロットを判断する標準品としても用いられ得る。治療剤の効果が測定されると、基準データセットは、作用因子の投与前に採取された遺伝子発現プロファイルに対応し得る。新しく製造された製品の品質管理が決められると、基準データセットは、その製品の貴重な標準品に一致し得る。しかし、所望の適当な規格化技術が使用され得る。例えば、平均基準プロファイルデータセットは、自然で育ったハーブの栄養物からなる本物の材料から得られ、放出のために調製された多くの化合物中での精度又は精度の欠如を示すため、時間がたちそして異なるロットで比較される。

【００６８】

較正データ

一つの較正データセットは、一構成要素の一つの一次プロファイルデータセットとパネルにおける所定の遺伝子座についての相当する一構成要素の基本的標準プロファイルデータの関数として記載され得る。例えば、較正されたプロファイルデータセットは、治療処置のような介在を含む環境における細胞試料におけるパネルの構成要素について転写された又は特定の時間におけるＲＮＡの量（１つの一次プロファイルデータセット）の、その試料とはある態様で異なる細胞における同じパネルの構成要素について転写されたＲＮＡの量（一つの基本的標準プロファイルデータセット）に関する比を計算することにより誘導され得る（図５及び図６）。本願発明者らは、繰り返し試験された試料において、較正されたプロファイルデータセットが再現されることを見出した（図１７）。又、本願発明者らは、生体外において被験体からの試料が化合物にさらされたときに得られる較正されたプロファイルが、生体内における試料にさらされた試料からの較正されたプロファイルに匹敵することも見出した（図１４及び図１６（ａ）、（ｂ））。又、本願発明者らは、作用因子で処理された指標細胞ラインが、生体内又は生体外細胞の集団から得られる、較正されたプロファイルデータセットに匹敵する較正されたプロファイルデータセットを与えることを見出した。さらに、本願発明者らは、被験体からの試料を指標細胞に投与することにより、被験体の健康、疾病状態、治療処理、老化又は環境による刺激又は毒素を含む被験体の生物学的条件に関する有益な較正されたプロファイルデータが提供されることを見出した（図２５）。

【００６９】

一つの較正されたプロファイルデータセットの好ましい使用は、被験体の生物学的条件を評価することである。それは、臨床的異常の診断又は予知の目的のためになり得る。健康状態又は年齢状態又はボディーマス又は個々の被験体が彼らのなかに見出し得る条件もしくは状態を示す、較正された一つのプロファイルデータセットを得ることが望ましい。例えば、その生物学的条件は、身体活動、調節又は運動、精神状態、投薬、食餌、又は放射能もしくは環境汚染もしくは感染性作用因子に対する地理的位置もしくは暴露、生物学的もしくは環境的毒性物質に関する。健康又は逆に臨床的異常が評価された場合、プロファイルの周期的又は定期的比較により健康状態における変化をモニターするために、較正されたプロファイルデータセットが用いられ得て、その臨床的異常は、炎症、自己免疫疾患、退行性疾患、アレルギー、血管疾病、虚血、発育疾患、ホルモン条件及び感染性疾患を含む複数の遺伝子が関与する可能性がある複合疾患作用であり得る。臨床的異常には、さらに、関節炎、喘息、多発性硬化症及びパーイメノポーザル変化（ｐｅｒｉｍｅｎｏｐａｕｓａｌ　ｃｈａｎｇｅｓ）が含まれる。生物学的条件は、呼吸、血管、神経、代謝、泌尿器、生殖、生態構造及び免疫系統又は他の代謝的状態を含む被験体の系統に影響を与え得る。生物学的条件の上記の例は、例示的に記載したものであり、限定されることを意図しない。

【００７０】

同様に、較正されたプロファイルデータセットは、感染性物質を特定するか、感染の継続期間、暴露の程度を評価するか治療の決定をする目的で感染性物質に対する宿主の応答を測定する、モニターする、又は予知するのに用いることができる。

【００７１】

作用因子の活性の評価には、一連の較正されたプロファイルが必要であり得る。本明細書において、較正されたプロファイルデータセットが、単一の化合物であるか、又は機能性食品もしくはハーブのような複合化合物であり得る作用因子の生物学的活性を示すのに用いられることができることが示されている。その作用因子は、指標細胞、生体からの細胞集団を用いることにより又は生体内投与により検定され得る。それらの検定は、一連の徴候パネル又は異なる生物学的条件についての拡大されたパネルに依存し得る。次に得られた較正されたプロファイルは、試験管内研究から、可能性のある生体内活性を推断するのに用いられ得る。作用の毒性及び機構への洞察も、較正されたプロファイルデータセットから推断することができる。例えば、ハーブのエキナシアは、体系的には両者とも測定されていないが、免疫刺激性及び抗炎症性を有すると考えられる。本願発明者らは、これらの及び他のハーブの生物学的活性を研究するための体系的アプローチを提供する。本願発明者らは、その作用因子を有する指標細胞ラインＴＨＰ－１又は抹消血管細胞を処理する効果を非処理の細胞と比較することにより、そのハーブの真偽の不確かな免疫刺激特性を研究した。非処理の細胞には、ＬＰＳ刺激の非処理細胞が含まれる。基本的標準プロファイルデータセットとその化合物での実験的処理との間の遺伝子発現における違いを測定するために、基本的標準プロファイルとして非処理細胞が用いられた。基本的標準プロファイルデータセットには、単一の試料又は一連の実験からの平均値が含まれた。次に、得られた較正されたプロファイルデータセットを特定のハーブについての較正されたプロファイルデータセットのライブラリー又は／及び異なる作用因子又は条件に関するライブラリーと比較することができた。

【００７２】

先に記載していない作用因子について得られた情報から、同じ作用因子の他の群を試験するための価値のある標準として役立つために、任意に徴候プロファイルとともに徴候パネルが誘導され得る。

【００７３】

<u>較正されたプロファイルデータの計算結果及びコンピューター補助</u>

基本的標準及びプロファイルデータセットに関する関数は、好ましい態様において対数として表わされる比である。そのプロファイルデータセットは、スプレッドシートにおいて表わされるか、又はグラフにより、例えば棒グラフもしくは表形式で表わされ得るが、又

、三次元表示においても表わされ得る。好ましくは、構成要素をｘ軸において記し、対数目盛りをｙ軸に記す。較正されたデータセットの要素は、基本的標準に関して、遺伝子発現の相対的増大を表わす正の値として又は遺伝子発現の相対的低減を表わす負の値として表わされ得る。較正されたプロファイルデータセットの構成要素は、同様の条件下で被験体から採られた同様の試料に関してある範囲内で再現すべきである。例えば、較正されたプロファイルデータセットの構成要素は、同様の条件下で被験体から採取された同様の試料に関して、ある大きさの程度内で再生し得る。構成要素は、５０％以内で再生でき、より特定すると、２０％以内で再生できる。較正されたプロファイルデータの各構成要素は、量Ｄ［Ｄ＝Ｆ（１．１）－Ｆ（０．９）であり、Ｆは第二の関数である］より大きく異なる値を有する場合に生物学的に重要である。

【００７４】

それは、生物学的条件、作用因子の生物学的効力、処理条件に関して有益であり、集団と比較するための較正されたプロファイルセットをつくるのに用いられ、医薬試験のためのありそうな候補を特定するために用いられ得る、生物学的条件に関して診断的又は予知的である他の臨床的指標と組み合わせて用いられ、製造、試験及び市場での販売を通して医薬又は機能的食品の開発を導くために用いられ得るパネルにおいて調べられた複数の遺伝子座からの遺伝子発現における増大の、低減の及び変化なしのパターンである。

【００７５】

量的遺伝子発現から得られる数値データ及び基本的標準プロファイルデータセットに関して較正された遺伝子発現からの数値データは、データベースにおいて又はデジタル保管媒体において保管され得て、患者の健康管理をすることを含む目的で又は、臨床的試験を行うために又は薬剤を特徴付けるために引き出される。そのデータを、地理的遠隔地から集めて共同利用のために貯えるために、例えばＷｏｒｌｄ　Ｗｉｄｅ　Ｗｅｂ、ｅメールもしくはインターネットアクセスサイトによりネットワークにおいて又はハードコピーにより伝達し得る（図８）。

【００７６】

好ましい態様では、貯えられたデータには、基本的標準のプロファイルデータセットの使用による変換の前のなまの遺伝子発現プロファイルデータ（一次プロファイルデータセット）及び例えば、基本的標準のプロファイルデータセットが特定の徴候パネルから誘導されているか否かに関する注釈並びにそのデータの解釈及び使用を容易にする他の注釈を含む較正されたプロファイルデータをつくるために用いられる基本的標準プロファイルデータセットの記録が含まれる単一の又は複数のデータベースにおいて記述的記録が貯えられる。

【００７７】

そのデータは、万国共通形式であるので、データの取り扱いがコンピューターを用いて容易に行われ得る。そのデータは、コンピューターで行われるアウトプットを提供するように組織化される。そのデータは、較正されたデータセットのグラフによる表示に任意に相当するアウトプットを提供するように組織化される。例えば、被験体から得た別個の試料を少なくとも１つのＲＮＡ又は蛋白質をP_1と表わし得る。その一次プロファイルデータセットは、M_j（M_jは個別のＲＮＡ又は蛋白質構成要素の量的尺度である）から成る。記録R_iは、ＭとＰの比であり、例えば、年、食餌、人種、性別、地理的位置、医療的異常、精神的異常、薬物療法、身体活動、ボディーマス及び環境的暴露に関する被験体における付加的データで注釈が付けられ得る。さらに、データ取り扱いには、さらに、現在、較正されたプロファイルデータベースとともに保持されていない付加的医療データを含有し得る二次条件データベースからのデータへのアクセスが含まれ得る。このような状況において、データクセスはコンピューターネットワークによりなされ得る。

【００７８】

コンピューターにおける先に記載したデータ貯蔵は、ユーザーによりアクセスされ得る形態において情報を提供する。従って、ユーザーは、情報をダウンロードすることを含む二次アクセスサイト情報をロードし得る。しかし、アクセスは、その中に含有される医療的

記録を保護するために、パスワード又は他のセキュリティーディバイスを有するユーザーに限定され得る。本発明のこの態様の特徴は、新しい又は注釈を付けた記録をデータセットに付加するユーザーの能力であり、記録が生物学的情報になる。

【００７９】

薬剤のような製品に関する較正されたデータセットのグラフによる表示は、較正されたプロファイル、より特定すると徴候プロファイルによる製品を標準化するための機械を提供する。そのプロファイルは、医薬品を販売促進するための特徴として用いられ得る。

【００８０】

本発明の種々の態様は、コンピューター装置とともに使用されるためのコンピュータープログラム製品としても実施され得る。その製品は、一次プロファイルデータを得るために又、較正されたプロファイルを生成するためにプログラムコードを含み得る。そのような実施には、コンピューターで読み取り可能な媒体（例えば、ディスケット、ＣＤ－ＲＯＭ、ＲＯＭ又は固定ディスク）のような実態的な媒体、又は媒体上のネットワークに接続させるコミュニケーションアダプターのような変復調装置又は他の接続器によりコンピューター装置に伝達される一連のコンピューター使用説明書が含まれる。その媒体は、実態的媒体（例えば、光学式又はアナログ式のコミュニケーションライン）か又は無線技術で実行される媒体（例えば、マイクロ波、赤外線もしくは他の伝達技術）であり得る。一連のコンピューター使用説明書は、好ましくは、その装置に関して本明細書に先に記載された機能性のすべて又は一部を具体化する。当業者は、そのようなコンピューター使用説明書が、多くのコンピューターアーキテクチャー又はオペレーティングシステムでの使用のためにいくつかのプログラム言語で書かれることができることを認識しなくてはならない。さらに、そのような使用説明書は、半導体の、磁気の、光学的又は他の記憶装置のようないずれかの記憶装置に入れ得て、光学的、赤外線、マイクロ波又は他の伝達技術のようないずれかのコミュニュケーション技術を用いて伝達され得る。そのようなコンピュータープログラム製品は、コンピューター装置でプレインストールされている（例えば、オンシステムＲＯＭ又は固定ディスク）か又はサーバーからもしくはネットワーク上の電子掲示板（例えば、Ｉｎｔｅｒｎｅｔ又はＷｏｒｌｄ　Ｗｉｄｅ　Ｗｅｂ）から配布された印刷された又は電子的文書化物（例えば、収縮包装にされたソフトウエア）を添えた移動媒体として配布され得ることが期待される。又、コンピューター装置はさらに、一次データセット及び較正されたプロファイルデータセットを得るための引き出しモジュールを含んで供給される。

【００８１】

臨床試験

臨床試験を行うための較正されたプロファイルデータセットの使用を、臨床試験を行うための又は患者のケアをするための先に記載した方法及び操作を用いて図１０に図解した。さらに、リポ多糖類のような公知の刺激物質により刺激されるＴＨＰ－１のような特定の指標細胞ラインを用いることにより、実験室間の標準化を行い、得られたプロファイルが、実験室が正確に臨床試験計画案を行う尺度として作用する。

【００８２】

臨床試験を補うために用いられる本発明の態様をいかに用い得るかの例には、被験体の選択のための新規な方法を提供することが含まれる。所定の生物学的条件についての予備決定された最適な較正されたプロファイルにより候補被験体が含まれるか又は除外される臨床試験により、他の方法で可能なよりも正確にモニターすることがもたらされる。又、例えば、複雑な因子又は条件を有する不適格な被験体をふるいにかけて除外するので、臨床試験の計画においてより大きな効率がもたらされる。較正されたプロファイルデータは、又、二重盲検プラシーボ研究から非応答者を除くことにより、「シグナル対ノイズ」を増大する。遺伝子発現プロファイル作成を用いる臨床試験計画の基本構造は、いくつのフォーマットのいずれかに従う。それらには、新しい治療薬に対する生体外試験における候補被験体からの体液を試験し、予備決定されたパネルを用いて作用因子処理された試料及びプラシーボ処理された試料に関する較正されたプロファイルを分析し、候補被験体が試験

された組成物に対する副作用なく応答し得るか否か評価することが含まれる。選ばれた適応症において、細胞が対象被験体又は他の被験体又は確立された細胞ライン又は対象被験体から取り出された細胞試料から派生し、細胞試料が血液、尿、精液、羊膜もしくは脳脊髄液試料を含む体液、又は口腔、眼、鼻、膣からのような粘膜からの削りとったものから又は上皮、肝臓、胸骨髄、精巣を含む生検材料による又はいずれかの部位における腫瘍から外科的に取り出した腫瘍組織から得られ得る、試験管内細胞培養物又は生物体培養物から得られたプロファイルデータが望ましい。

【００８３】

指標細胞ライン又は生体外で試験される被験体の試料から得られる較正されたプロファイルデータセットを用いる試験管内投薬量及び毒性研究は、臨床試験の開始前の有用な情報を提供することができ、有益な効果の存在を確認できる可能性を増大しながら臨床試験の費用及び時間を有意に低減させることができる。特に、治療結果における影響を最大にするために個別的基準で投与量を最適にすることができる。例えば、図１２は、いかに生体外血球がＬＰＳの刺激効果及び抗炎症薬（メトトレキサート、メクロフェナメート又はメチルプレドニゾロン）でのその後の処理に応答するかを示す。そのデータは、いかにメトトレキサート又はメクロフェナメートの効果が、基本的標準がＬＰＳ処理血液である同様の較正されたプロファイルデータを生じるかを示す。一方、メチルプレドニゾロンは、他の二つの化合物とは実質的に異なる効果を有する。生体外のＬＰＳ刺激血液に、イカイナシア（Ｅｃｈｉｎａｃｅａ）、ウサギキク属（Ａｒｎｉｃａ）及びシベリアンヤクヨウニンジン（Ｓｉｂｅｒｉａｎ　Ｇｉｎｓｅｎｇ）が適用されたときに得られる較正されたプロファイルが比較された図２１に図解されているように、複合混合物での同様の種類の分析を行うことができる。この例において、すべての３つの作用因子が単一の被験体からの試料に関して各々異なって作用するようである。非既知の対象、又は複合又は単一の化合物への活性もしくは代謝パターンを有する化合物を既知の又は予備決定されたプロファイルと比較するために同様の分析を用いることができる。

【００８４】

臨床試験の計画及び実施において又は補助的手段として上記の方法及び操作を用いることができる。さらに、上記方法及び操作は被験者の健康及び臨床試験の前、間、及び後の作用因子への被験者の応答性をモニターするのに用いることができる。それには、複数の作用因子が互いに干渉するか否か、拮抗的又は付加的に作用するか否か、又は互いに関して毒性であるか中性であるか否かをモニターすることが含まれる。この種類の情報は、個体が、増加する数の薬物療法を受けているときに非常に重要である。

【００８５】

同様に、上記の方法及び操作は、個体についての又は集団についての被験者のケアを管理するために用いられ得る。そのような方法及び操作は、較正されたプロファイルセット、及び研究又は試験を行うために得られたデータベースを用いる地域的又は世界的研究ネットワークを開発するためにも用いられ得る。

【００８６】

グラフ形式における較正されたプロファイルデータ及び関連するデータベースの両者は、その両者から抽出される情報とともに、種々の目的のために一緒に又は別個に販売される商品である。例えば、較正されたプロファイルデータセットのグラフによる表示は、その製品を販売促進し得るその活性に関するその製品の記載を提供する。又は、較正されたプロファイルデータセットのグラフ及び基本的標準プロファイルデータベースへのアクセスは、製造業者のために価値ある標準に対する製品の個々のバッチを試験する手段を提供する。

【００８７】

そのデータは、臨床試験を計画するために戦略的に使用されることができる。又、遠隔地において患者に個人的なヘルスケアを提供することを実施する医師にとって有用であることができる。従って、医師は、特定条件の処理の前及び後に、較正されたプロファイルデータセットのために個別化したデータベースを設定し得る。被験体における新しいデータ

を、医師への各来診における個別化したデータベースに加えることができた。そのデータは、患者からの試料における一次プロファイルデータセットを得ることを医師に可能にさせるキットの使用により遠隔地において生じ得る。遠隔のユーザーがそのサイトにアクセスするために、基本的標準データセット及び較正されたプロファイルデータセットのライブラリーを含み、特定の基準により分類され、単一の個体より大きな集団からのデータを表わす世界的なネットワークへの保証されたアクセスが必要であることが意図される。世界的なデータベースへのアクセスは、パスワードで保護され得て、それにより変造記録からデータベースを保護し、個人的な医療データを保護する。特定の個体に起こり得る毒性作用及び他の種類の医薬相互作用を完備した、薬物類の化合物のカタログを作成するために、較正されたデータセットにより与えられるグラフ形式が用いられ得る。

【００８８】

世界的データベースへのアクセスには、選ばれたデータを二次的アクセスサイトにロードするオプションが含まれ得る。このプロセスには、どのサイトがユーザーにより望ましいかの情報をダウンロードすることが含まれ得て、情報のハードコピーを保証することが含まれ得る。データベースの保全を維持するために、いかに及びどのデータがオフロードされるか又はコピーされるかを制御することが望ましい。臨床データの世界的なネットワークが情報源でありながら、疫学的研究及び作用因子の作用の機構に関する研究及び較正されたプロファイルデータセットにより決定されるときの個体間の変動性の本質についての研究を含み得る研究を行うことにおける有用性を有することが意図される。

【００８９】

医療用途の例

（ａ）感染性疾病の初期検知：感染に対する、例えば、肝炎ウイルスのようなウイルスに対する、又はマイコバクテリウム・ツベルクロシス（Ｍｙｃｏｂａｃｔｅｒｉｕｍ ｔｕｂｅｒｃｕｌｏｓｉｓ）（結核のグラム陽性作用因子）のような細菌に対する初期の又は即時の応答を示すヒトにおける遺伝子発現を測定するために、マウスからのマーカー又は代理マーカーを得る（図４）。候補遺伝子を特定し、攻撃の存在下でのそれらの遺伝子の発現における変化により一組のマーカーを与える。その一組のマーカーは、被験体のゲノムによりコードされたマーカーと感染性作用因子のゲノムによりコードされたもう１つの個別のマーカーを組み合わせ得る。例えば、ウイルスの即時の初期の遺伝子例えばウイルス複製の酵素をコードする遺伝子、並びにＩＬ－２、ＩＬ－４及びＩＬ－５のいずれか又はすべてについての遺伝子のような宿主遺伝子の発現における変化は、医療症状の始まりの前の条件を検知することができる医療条件についてのマーカー又は代理マーカーを含み得る。この方法は、現在の診断技術を用いて可能であるよりも感染のより早期の検知を提供する。

【００９０】

（ｂ）試験管内検定及び生体内検定から得られる毒性プロファイル及び機構的（ｍｅｃｈａｎｉｓｔｉｃ）プロファイル

集団の細胞への化合物の投与から生じる毒性及び機構的情報を、較正されたプロファイルデータセットを用いてモニターすることができる。下記は、この情報を得るための実験計画の例である。最初に、実験群を確立する：（１）治療物質を用いずにかつ刺激を用いずに維持された対照細胞。（２） 治療物質で処理された、しかし刺激を用いずに維持された細胞。（３） 治療物質を用いずに、しかし刺激を用いた細胞。（４） 治療物質を用い、かつ刺激を用いた試料。細胞の集団を、本技術分野でよく確立された方法を用いて培養プレートにおいて生成した一次細胞培養物、又は全血からの成熟した分化した細胞調製物又は対象生物、この例ではマウス、からの単核細胞から選ぶことができる。

【００９１】

細胞を、グラム陰性細菌から精製されたＬＰＳ［病原性細菌、例えば、ダーニス菌（Ｓａｌｍｏｎｅｌｌａ ｔｙｐｈｉｍｕｒｉｕｍ）から及び大腸菌Ｏ１１５７：Ｈ７からのＬＰＳ調製物は、ミズーリー州、セントルイスのＳｉｇｍａから入手できる。］での予備処理により目的とする生理的条件を与えるために刺激される。この例において、細胞試料に

投与される治療物質は、疾病原因における鍵であることが知られている酵素の阻害物質、すなわち、プロテアーゼ又は核酸ポリメラーゼの阻害物質である。治療物質の添加による処理及びさらに４乃至６時間のインキュベーションの後に、その細胞の試料を回収し、遺伝子発現のために分析をする。核酸、特にＲＮＡは、本技術分野で公知の方法により試料から調製される［例えば、Ｌｙｓｅ－Ｎ－Ｇｏ（商標）（イリノイ州、ロックフォードのＰｉｅｒｃｅ Ｃｈｅｍ. Ｃｏ.を参照）。試料を、量的複製操作［量的ポリメラーゼ鎖反応操作（ｑｕａｎｔｉｔｉｖｅ ｐｏｌｙｍｅｒａｓｅ ｃｈａｉｎ ｒｅａｃｔｉｏｎ Ｐｒｏｃｅｄｕｒｅ）（ＱＰＣＲ）］によるＱＰＣＲにより分析される（例えば、Ｇｉｂｓｏｎ, Ｕ. １９９６ Ｇｅｎｏｍｅ Ｒｅｓ. ６：９９５－１００１及びその中に引用された文献を参照）。一般的なプライマーを用いて全ＲＮＡを評価する。細胞についての作用因子の毒性を、生体染色取り込み、ＤＮＡ合成の速度（染色された細胞と比較した標識された核酸の放射能写真技術）、ＤＮＡ特異性アイズ（ＤＮＡ－ｓｐｅｃｉｆｉｃ ｅｙｅｓ）による染色（Ｈｏｅｃｈｓｔ）等により、非処理細胞において測定され得る。機械作用プロファイルは、デ・ノボ（ｄｅ ｎｏｖｏ）のアップレギュレーションされた又はダウンレギュレーションされた遺伝子の識別物質により決定され得る。さらに、治療物質の存在下で、いくつかの遺伝子は発現されず、ＬＰＳによる刺激の効果を抑制することにおける治療物質の潜在的効力を示している。例えば、図２１において、ＬＰＳ＋イカイナシア（Ｅｃｈｉｎａｃｅａ）の存在下でいくらか刺激されるＩＣＥの量は、ＬＰＳで刺激された細胞が存在しない作用因子に比例してＬＰＳ＋ウサギキクにより実質的に低下する。ＬＰＳ＋イカイナシア（Ｅｃｈｉｎａｃｅａ）の存在下で低下するＨＳＰ７０の量は、ＬＰＳで刺激された細胞が存在しない作用因子の添加に比例してＬＰＳ＋ウサギキク及びＬＰＳ＋シベリアンヤクヨウニンジンの存在下で実質的に刺激される。ＬＰＳ＋イカイナシア（Ｅｃｈｉｎａｃｅａ）の存在下でわずかに増加するＩＬ－１２ｐ４０の量は、ＬＰＳ刺激に比例してＬＰＳ＋ウサギキク属及びＬＰＳ＋シベリアンヤクヨウニンジンの存在下で実質的に低下する。図１６は、ウサギキク属＋ＬＰＳ、又はなし＋ＬＰＳと比較したときにプレドニゾロンの存在下で、ＩＬ－１ａ、ＩＬ－１ｂ、ＩＬ－７、ＩＬ１０、ＩＬ－ＩＬ－１５、ＩＦＮ－ｇ、ＴＧＦ－ｂ、ＴＮＦ－ｂ ｃｏｘ－２及びＩＣＡＭについての全血における遺伝子発現の非常に増大された低下を示す。

【００９２】

（ｃ）他の組織又は器官における毒性を予測するための血球における遺伝子発現の定量

他の器官、例えば肝臓における病理学的条件の出現を評価する目的のために、被験体の血液試料から白血球を得ることができる。白血球において発現した遺伝子、例えば、一組のリンフォカイン及びサイトカインをコードする遺伝子のプロファイルデータセットを得る。相互関係、例えば他の被験体に対する相互関係及び治療物質の投与前の被験体に対する相互関係を調べるために、そのデータセットを、データベースのデータセットと比較する。

【００９３】

この方法により、例えば、アセトアミノフェン［チレノール（Ｔｙｌｅｎｏｌ）］の投与と、この治療物質に対する感受性との間の相互関係が引き出され、肝臓の損傷により明らかにされる。肝臓に対する現実の損傷の始まりの前に検知される治療物質感受性の初期の予測は、臨床的に利用でき、被験体はアセトアミノフェンのさらなる投与を受けない。データベースの成功は、ビリルビンにおける増加又は肝臓病理学の他の兆候のような伝統的医療評価を始める前に１つの又は複数の相互関係を検知する能力である。

【００９４】

（ｄ）自己免疫疾病の治療における副作用の深刻さの予測及び副作用の予測のための、血球からの較正されたプロファイル

自己免疫性疾病、例えば慢性関節リウマチの症状の始まりの見込み及びタイミングは、マーカー及び代理マーカーの遺伝子発現プロファイリングと先に記載したプロファイルデータベースとの比較の方法により決定されるマーカー及び代理マーカーの発現の様相により、モニターし得る。このように、差し迫った始まりの表示が得られ、始まりを防ぐために

予防的処置の利用によって事前の管理がとられ得る。さらに、ユーザーは、一組の潜在的治療物質を選ぶことができ、被験体に治療の全過程が与えられる場合、全過程の前に、所定の作用因子について被験体が副作用を示す見込みを評価することができる。例えば、本発明の態様を用いて、作用因子、メトトレキサートの一投与量が、関節炎を有する被験体に、かつ、治療物質を必要としているときに投与され得る。メトトレキサートの一投与量に応答した被験体の遺伝子発現プロファイルデータが、この作用因子に対して副作用を有する被験体からのデータセットに相互関係を有する場合、全過程のメトトレキサートの投与は指示されない（ｃｏｕｎｔｅｒｉｎｄｉｃａｔｅｄ）。逆に、遺伝子発現プロファイルデータセットが、メトトレキサート治療の過程の投与にプラスに応答した被験体のものと相互関係を有する場合、この治療物質は、副作用のずっと低い確率を有するその被験体に投与されることができる。

【００９５】

図面の説明

図１－４は、較正されたプロファイルデータセットのいくつかの応用を示す。図は３つの可能な計画を示す。第一に、候補の治療物質が、その分子の薬理学及び毒物学を決定するために試験され得る。その試験は、その薬剤についてどの活性が予測されるかを基礎として一連のパネルについての較正されたプロファイルデータセットを得ることが含まれる。その治療物質に暴露された細胞の集団は、マウスによって表わされた生体内投与の結果、又はその細胞が指標細胞ラインもしくは被験体からの生体からの試料である試験管内での直接暴露の結果であり得る。そのスクリーニングの結果は、ヒト被験者において試験するための、より効果的な薬剤候補の識別である。

【００９６】

図１における第二の計画は、潜在的治療物質をスクリーニングするための適する臨床的集団を識別するための較正されたプロファイルデータセットの使用である。毒性がないことを示すこと及び臨床的効力を示すことの両方には、臨床的集団についてのある仮定条件が必要である。較正されたプロファイルデータセットは、臨床試験のために選ばれた個体の生物学的条件についてのそれらの仮定条件を確立するための手段を提供する。

【００９７】

図１における第三の計画は、特定の条件の予知又は診断をさせるように徴候パネルを用いてその変化を識別できるような健康の状態の個体における較正されたプロファイルデータセットのアーカイブを創製することを含み得る、個別化された医療を実行するための機会である。さらに、患者についての較正されたプロファイルデータセットについての貯えられた情報は、患者にとって最も有効でありそうな可能な治療剤の群の１つを選ぶこと、薬剤の用量を最適にすること及び症状が起こる前に薬剤の相互作用により起こり得る副作用を検知することを可能にさせる。

【００９８】

被験体の生物学的条件を評価するための先に記載した新しいてアプローチは、図２－４に示されているように、ある生物学的条件における作用因子の効果を測定するための生体からの又は試験管内検定に適用され得る。被験体からの試料は、生体外で直接測定され得るか又は被験体における効果を予測するために作用因子に対して生体外で試験される。それにより、特定の条件を処理するのにすべてが用いられ得る一部類の薬剤中から選ばれる、どの薬剤が与えられた被験体にとって最も有効であり得るかを決定する迅速で効果的な方法が提供される。又、ある部類の個体において治療的能力の量的尺度を与えることができる指標細胞ラインにおいて、作用因子が試験され得る。

【００９９】

図２は、候補薬剤を見出すために候補化合物のライブラリーをスクリーニングすることに、いかに較正されたプロファイルデータセットが補助し得るかを示す。例えば、５００の候補薬剤を用いて開始し、それらは指標細胞又は生体からの体液もしくは組織においては徴候パネルに対して、試験管内毒理学又は代謝性指標物質について試験される。図２は、開発プロセスのみの後期段階に入り、有害な生物学的な相互作用により最終的には拒絶さ

れる、多数の化合物を示す。較正プロファイルデータセットの使用の初期採用は、成功しそうな候補をより容易に識別し、それによって失敗することが予測され得た化合物についての動物及びヒト実験の費用及び不適当な作用を低減させることが期待される。

【０１００】

図３は、化合物が、マウスのような実験動物に又は指標細胞ラインに投与され得る複数のスクリーニングを記載する。生体内又は生体からの又は指標細胞の試料は、さらに刺激物質により処理され得る。次に、その化合物及び前記刺激物質の両方の結果を毒性又は機械作用について徴候パネルプロファイルを用いて検知し、薬剤なしで＋／－刺激、又は＋／－薬剤で刺激なしの効果を比較する。試験管内（左パネル）及び生体内（右パネル）研究は、化合物（薬剤、機能性食品、環境刺激物質）の作用を評価するために用いられ得る。右側パネルは又、生体内における被験体の同様の処理の結果を予測するために、「試験管内臨床試験」の特定の態様、すなわち、被験体から得られ、試験管内で（又は生体外で）化合物（刺激物質あり又はなし）で処理された細胞の処理を示す（特定の例についての図１５を参照）。両パネルからの結果が、毒性及び機構的プロファイルとして記載される。潜在的毒性を、例えば毒性パネル又は肝臓代謝パネルを用いて、評価するために、かつ、作用の分子機構を示し、かつ、区別化する遺伝子パネルの決定的な選択により作用のあり得る機構を決定するか又は確認するためにどちらかの実験過程が用いられ得る（特定の例についての図１２を参照）。

【０１０１】

図４は、細胞が被験体から採取され、化合物及び攻撃又は刺激物質も添加して生体外で試験される生物学的検定を示す。刺激物質がリポ多糖類（炎症性物質）であり、薬剤がメトトレキサート又はメクロフェナメート又はメチルプレドニゾロンのいずれかであり、炎症についてのパネルを用いて、ヒト被験者から採取された全血における刺激物質及び次に薬剤の生体外効果を図１２に示す。ＣＯＰＤ（慢性閉塞性肺疾患）の急性悪化治療及びこの疾病の慢性管理に通常用いられる薬剤であるメチルプレドニゾロンは、非特異的抗炎症作用因子によって効力が強いと考えられている。しかし、図２２に示されているように、遺伝子発現におけるその効果は、刺激物質によって変わる。それらの３つの刺激物質にわたる遺伝子発現における効果間の一般的な質的同様性があるが、グルココルチコイドの介在が保証されるときに、理解において重要であり得る量的差及び質的差の両方がある。

【０１０２】

本発明の態様により、量的遺伝子発現を測定するのに指標細胞集団が用いられ、試料物質における作用因子の効果は、情報を与える指標細胞ラインの選択に影響を与え得る。例えば、ＴＨＰ－１のような複製された細胞ライン又は一次細胞集団（末梢単核細胞）は、生体試料から直接得られたものに匹敵する情報を与え得る（図１５参照）。遺伝子発現の正常な状態は、０又はいくつかの写しから10^5又はそれより多い写しの範囲であり得る。

【０１０３】

同様に、その作用因子を投与し、選ばれた条件下での細胞について較正されたプロファイルデータセットを決定することにより、生体内、生体外又は試験管内のいずれかの細胞の集団における作用因子の効果について作用因子を評価し得る。このアプローチの例は、図１０－１６及び１８において与えられる。図１８はさらに、選ばれた構成要素が用量とともに変わり、従って、生物学的条件に関して予測された効果が変わることを示す単一の作用因子の異なる濃度についての較正されたプロファイルデータセットを与える。

【０１０４】

生物学的条件を決定する先の記載は、以下のように例示される。抗炎症性に関して医薬品又は機能性食品の作用を測定する。その効果の測定は、１つのパネルの構成遺伝子座、例えば、インターロイキン１α（ＩＬ－１α）又は腫瘍壊死因子α（ＴＮＦ－α）を含む炎症パネルを用いて確立される。抗炎症効果は最初に、指標細胞又は生体からの試料細胞を公知の炎症誘導物質（例えば、リポ多糖類又は他の誘発物質）で処理し、続いて、適する遺伝子座からの発現を抑制する又は低減することが予測される実験作用因子又は条件での処理をすることにより確立される。基本的標準プロファイルデータセットは、構成要素の

特定のパネルについての遺伝子発現におけるデルタ変化である。潜在的抗炎症作用因子の添加は、第一のデルタ変化に載せられた第二のデルタ変化をもたらす。そのことは、例えば図１２において示される。メチルプレドニゾロンは、基本的標準データセットがＬＰＳで刺激された細胞である場合、ＬＰＳで刺激された生体からの血球での、ＩＬ－２における実質的にダウンレギュレーションされた効果を有する。この場合は負のデルタである。一方、ＩＬ－２は、基本的標準データセットが刺激されていない細胞である場合、予めＬＰＳに暴露されていない全血においてはアップレギュレーションされた効果であるようだ（図１６ｂ）。このことは、メチルプレドニゾロンがＩＬ－２生成を刺激したという判断と一致する。

【０１０５】

被験体の生物学的条件の決定には、被験体についての付加的なデータを測定すること及び貯えることが含まれる。例えば、被験体がヒト被験者又は哺乳動物被験体である場合、付加的な臨床的指標は、血液化学、尿検査、Ｘ線、他の化学的検定及び物理的又は社会学的研究結果から決定され得る。

【０１０６】

図７は、較正されたプロファイルデータセットの集積によりデータベースの予測能力がいかに改良され、それによって生物学的条件又は作用因子についての情報を生ずることにおいてその価値を増すかを示す。

【０１０７】

図７は、例えば、治療介在の過程を予測し、集団、ありそうな代謝の機構もしくは作用の分子機構の予測又は、単一のプロファイルの、較正された精密な徴候プロファイルの収集物との比較を可能にする幅広いデータベースと比較した個体の被験体の過程に従うためのその予測能力に関してそのデータベースの使用を示す。

【０１０８】

いかにそのデータベースを用い得るかの好ましい態様が図８に与えられている。図８、情報源データベースからのデータプロファイルセットの表示を示す。インプットの登録事項には、名前、実験種類、その登録が新しい参照；細胞／組織／種であるか否か、新しい治療作用因子（化合物）、用量及び付加的なパラメーターであるか否か及びその治療物は新しいか否かが含まれる。遺伝子（新しい遺伝子）及び蛋白質（新しい蛋白質）の同一性による観察が記録される。刺激物質又は、あれば他の処理、及び用量が登録される。遺伝子（及び／又は蛋白質）発現、発現値、適している場合、発現単位及び発現時間が示される。図８は、複合天然生産物から、ヒトにおける臨床試験までの、適用できる研究分野の範囲、測定の伝統的な形態への関連、及び文献引用のような評価、臨床的指標、及び伝統的薬物動態学測定値を示す。次に、そのデータベースが含まれている精密なプロファイルの熟練者の分析が、製品開発及びマーケティングを導くために用いられ得るか又は単一の個体又は個体の集団の健康に関する臨床意思決定を改良するのに用いられ得る。

【０１０９】

記録の１つの形態が、アイデンティティー、伝統的医薬／医療データを含む医療歴、文献データから決定される医療適応症、データベースにおける分析の付加的種類への照会に関する被験体又は作用因子についての情報を提供することが予測される。

【０１１０】

図９は、プロファイルデータが、ネットワークでリモートにアクセスされるデータベースからのデータを用いて評価される、本発明の態様を示す。図９は、そのデータが１つ以上の記憶位置から引き出され、中央データベース及び得られた情報を用いて比較され、例えば、個体の又は集団の処理の過程に影響を与えるために用いられることが予測されることを示す。１１０９の、２方向いずれにも送られる性質は、それによってデータベースが処理又は開発の過程に影響を与え、そのような介在に対する結果又は応答が再び、データベースの一部になる、反復するプロセスを示す。第一の場所において、図５におけるように、ボックス１１０１において得られた組織試料から、ボックス１１０２に拠った複数のＲＮＡ種が誘導され、次にボックス１１０３において、プロファイルデータが、ボックス１

１０１において得られる組織試料に直接関係があるプロファイルデータセットを出すために量化される。プロファイルデータセットを評価するために、ボックス１１０４において情報が、第二の場所にあるデータベース１１０８から引き出される。実際、そのデータベースは、多数の場所と情報伝達し得て、その各々の場所が、評価されるに違いないプロファイルデータを発生する。データベースからの情報の取り出しは、本技術分野で公知の方法でネットワーク１１０９で行なわれ、そのネットワークはインターネットを含み得る。データベース１１０８から情報が得られたら、その情報は、被験体の医療条件が検定されるボックス１１０６における結果とともにボックス１１０５における定量化プロファイルデータを評価するのに用いられる。データベース１１０８は、ボックス１１０３において定量化されたプロファイルデータを反映するために、ネットワーク１１０９より最新のものにされているボックス１１０８に入っている。このように、すべての場所で得られたプロファイルデータを反映するために、データベース１１０８は、最新のものにされており、各々の場所は、すべての場所から得られるデータの利益を有する。

【０１１１】

実施例

例１.

ａ）薬剤により影響を受ける生物的状態の生体外評価に用いられる全血の用法

人の血液は静脈穿刺により得られ、少なくとも3時点につき十分な容量で基準（基線）に関して、無刺激及び有刺激でサンプルを等分することによってアッセイ（ａｓｓｅｙ）に備えられる。典型的な刺激物質としてはリポ多糖類（ＬＰＳ）、フィトヘマグリチニン（ＰＨＡ）及び熱死滅ブドウ球菌（ＫＨＳ）又はカラゲーニンがあり、個別的（典型的に）又は組合せて用いられ得る。ヘパリン化された全血の等量は刺激物質なしで混合され、３０分間５％ＣＯ２の雰囲気内で３７℃に保たれる。異なった濃度で刺激物質が加えられ、混合されかつ緩くふたをして３０分間３７℃に保たれる。この時点で追加の試験化合物が加えられ、試験化合物の期待される薬物動態に依存して異なった時間にわたり保持される。限定された時間で遠心分離により細胞が収集され、各種の標準手段によりプラスマ（血漿）が除かれＲＮＡ抽出される。

【０１１２】

ｂ）遺伝子発現測定用ＲＮＡ調製

核酸、ＲＮＡ、ＤＮＡは、試験集団又は指標細胞ラインの細胞、組織又は液体から精製される。ＲＮＡは各種の標準手順又はＲＮＡ分離戦略（Ａｐｐ．５５－１０４，ｉｎ RNA Methodologies, A laboratory guide for isolation and characterization, 2nd edition, １９９８．Ｒｏｂｅｒｔ Ｅ．Ｆａｒｒｅｌ．Ｊｒ．，Ａｃａｄｅｍｉｃ Ｐｒｅｓｓ）を用いて核酸混合物から選択的に得られる。現用法ではＡｍｂｉｏｎ（ＲＮＡｑｕｅｏｕｓ™，Ｐｈｅｎｏｌ－ｆｒｅｅ Ｔｏｔａｌ ＲＮＡ Ｉｓｏｌａｔｉｏｎ Ｋｉｔ，Ｃａｔａｌｏｇ ＃１９１２，ｖｅｒｓｉｏｎ ９９０８；Ａｕｓｔｉｎ，Ｔｅｘａｓ）からのフィルターを基礎とするＲＮＡ分離システムが用いられる。メッセージ特定プライマー又は任意（ランダム）プライマーを用いて特定のＲＮＡが増幅される。特定のプライマーは，人又は他の動物から得られる遺伝子及びｃＤＮＡライブラリーからの情報を含む公共データベース（ｅ．ｇ．，Ｕｎｉｇｅｎｅ，Ｎａｔｉｏｎａｌ Ｃｅｎｔｅｒ ｆｏｒ ＢｉｏｔｅｃｈｎｏｌｏｇｙＩｎｆｏｒｍａｔｉｏｎ，Ｎａｔｉｏｎａｌ Ｌｉｂｒａｒｙ ｏｆ Ｍｅｄｉｃｉｎｅ，Ｂｅｔｈｅｓｄａ，ＭＤ）から得られるデータで合成される。試験又は指標サンプルから得られる特定ＲＮＡを選択的に増幅するためにプライマーが選択される（例えば、ＲＴ ＰＣＲ，Ｃｈａｐｔｅｒ １５ ｉｎ RNA Methodologies, A laboratory guide for isolation and characterization, 2nd edition, １９９８．Ｒｏｂｅｒｔ Ｅ．Ｆａｒｒｅｌｌ．Ｊｒ．，Ａｃａｄｅｍｉｃ Ｐｒｅｓｓ；Ｃｈａｐｔｅｒ ２２ ｐｐ．１４３－１５１，ＲＮＡ ｉｓｏｌａｔｉｏｎ ａｎｄ ｃｈａｒａｃｔｅｒｉｚａｔｉｏｎ ｐｒｏｔｏｃｏｌ，Ｍｅｔｈｏｄｓ ｉｎ ｍｏｌｅｃ

ular biology, Volume 86, 1998, R. Rapley and D. L. Manning Eds., Human Press, or 14 in Statistical refinement of primer design parameter, Chapter 5, pp. 55−72, PCR application: protocols for functional genomics, M. A. Innis, D. H. Gelfand and J. J. Sninsky, Eds., 1999, Academic Press 参照）。増幅は、等温条件下又は温度循環装置を用いて行われる（例えば、ABI 9600 or 9700 or 7700 obtained from PE Biosystems, Foster City, CA; see Nucleic acid detection methods, pp. 1−24, in Molecular methods for virus detection, D. L. Wiedbrauk and D. H., Farkas, Eds., 1995, Academic Press）。増幅された核酸は、増幅プライマーにつき述べた公共データベースから同定及び合成される蛍光標識付検出プライマーを用いて検出される（例えば、Taqman（登録商標）, PCR Reagent Kit, Protocol, part number 402823 revision A, 1996, PE Applied Biosystems, Foster City CA参照）。本件では増幅されたDNAは、PE Byosystems （フォスター市、カリフォルニア州）から得られるABI Prism 7700 Sequence Detection System を用いて検出かつ定量化される。試験サンプルに含まれるか又は標識細胞ラインから得られる特定RNAの量は、観察される蛍光の相対量と関連付けられ得る（例えば、Advances in quantitative PCR technology: 5_ nuclease assays, Y. S. Lie and C. J. Petropolus, CurrentOpinion in Biotechnology, 1998, 9; 43−48, or Rapid thermal cycling and PCR kinetics, pp. 211−229, chapter14 in PCR applications: protocol for functional genomics, M. A. Innis, D. H. Gelfand and J. J. Sninsky, Eds., 1999, Academic Press参照）。

例2. 異なった炎症性刺激物質は異なった基準プロファイル（グラフ）データセットを発生させ、同一クラスの非炎症性内の異なった試薬に対して較正された精密なグラフは異なった徴候プロファイルに帰着するようにされる。

【0113】

図11は、試験されら3つの抗炎症性剤に対する較正された精密なプロファイルデータセットが異なった徴候グラフに帰着するように、異なった基準プロファイルデータセットを与えるための異なった炎症性刺激の有用性を立証する。異なったプロファイルは、単一クラスの治療的抗炎症剤から得られる3種の薬剤の作用の分子ターゲット及び機構上の差を反映する。同図は同様に較正器と比較すると、較正されたプロファイルにつき10倍差未満からプラス又はマイナス10E13までの遺伝子発現での増加又は減少に及ぶ異常な検出範囲（y軸）を例示する。較正器との比較では、較正された組から増加、減少するか又は変化しない遺伝子発現グラフに帰着する。

【0114】

図11a）は、熱死滅されたブドウ球菌（KHS）で刺激された細胞の相対的遺伝子発現及び3つの異なった化合物（TPCK, UT77及び“Dex”又はデキサメタゾン（炎症治療剤））の影響を示す。化合物TPCKは相対的IFN−γ発現の10倍減少及びIL−4及びIL−5発現の100, 000倍減少を与えた。さらに、化合物UT77は遺伝子符号化IL−5の相対的発現でさらにより大きな増加を与えると共にIL−1発現（10倍を越える）及びIFN−γではより適度な増加を与えた。このような効果は病気原因では高度に有意であり得ると共にこれらの化合物の治療剤又は同様に作用する化学的実体又は薬品としての有用性に関して断定的価値を有し得る。HKS細胞はグラム陽性バク

テリア感染の試験管内モデルである。

【０１１５】

図１１ｂ）は、グラム陰性バクテリア感染の試験管内モデルである、リポ多糖類（ＬＰＳ）処理された細胞の１２遺伝子発現の分析を表示する。これらのデータは、図１１ａ）データに対していくつかの著しい対照性を含む。従って、治療剤Ｄｅｘでの処理は、ＬＰＳ処理された細胞のＩＬ－２遺伝子の発現で著しい減少及びＨＫＳ処理された細胞のＩＬ－２発現で著しい増加を与えた。異なって刺激された細胞の遺伝子発現の著しく大きな差はＩＬ－４及びＩＬ－５遺伝子で見られ得る。対照的に、ＩＦＮに対する遺伝子の発現は、刺激及び任意の治療剤の双方で処理された細胞で同様に反応した。

【０１１６】

これらの基準によって、ＩＬ－２、ＩＬ－４及びＩＬ－５に対する遺伝子の発現は、グラム陽性及びグラム陰性バクテリア感染に対する細胞の反応を区別するために候補者標識（マーカー）又は代理マーカーと認められた。

【０１１７】

例３．特定の状態を処理する単一の治療剤は、遺伝子座の所与のパネルに対する徴候プロファイルによって特定の状態を同様に扱う第２治療薬剤から区別され得る。

【０１１８】

図１２は、炎症を含む生物的状態を示す８構成成分を有するパネルに対し較正されたプロファイルデータセットを示す。同プロファイルは、３つの異なった抗炎症性の薬剤メトトレキサート、メクロフェナメート及びメチルプレドニゾロンに対して示される。図示されるように各薬剤に対する較正されたプロファイルデータセットはその薬剤に対する徴候プロファイルを表わす。この徴候プロファイルは一バッチの薬剤に対する品質管理を確立する手段として役立ち得る。実に、市場におけるか又は開発中の化合物又は各クラスの化合物は徴候プロファイルによって特徴付けられ得ると考えられる。徴候プロファイルはグラフフォーマットで、さらに特定的には図１２に与えられる棒グラフとして表わされ得る。図１２では生体外サンプルが試験された。血液サンプルが被験体からとられた。同サンプルの等分された部分が生体外リポ多糖類（ＬＰＳ）にさらされた。３０分後、図示されるような抗炎症剤が血液サンプルの等分部分に加えられ、約４時間後遺伝子（Ｉｌ－ａ，Ｉｌ－２，Ｉｌ－８，Ｉｌ－１０，Ｉｌ－１２ｐ３５，Ｉｌ－１２ｐ４０，Ｉｌ－１５，ＩＦＮ－Ｇａｍｍａ及びＴＮＦ－ａ）のパネルの発現が決定された。メトトレキサートおよびメクロフェナメートの較正されたプロファイルは類似していたが、メチルプレドニゾロンの較正されたプロファイルは実質的に異なっていた。その差は抗炎症性化合物の一般クラス内のこの薬剤の作用の機構又は対象の差を反映するであろう。基準は、追加の薬剤が全く不在のリポ多糖類に対するプロファイルデータセットである。

【０１１９】

例４．適切に誘導されている多くの遺伝子座の全域に亘る遺伝子発現の測定から当該較正された精密なプロファイルが決定される場合を通して単一の個体内のプロファイルに関して比較的低い変異性が存在する。

【０１２０】

図１３ａ）ｂ）およびｃ）は、全血の２つの異なったサンプルに対する較正された精密なプロファイルデータセットのグラフ表現を示す。２週間以上離れた２つの別個の機会に普通の健康な単一ボランティアからヘパリン化された全血が収集された。サンプル９９１１１６に対する図１３ａおよびサンプル９９１０２８に対する図１３ｂは、３つの異なった薬剤の１つによる刺激に応答して２４集団のパネル（即ち、炎症パネル）を用いた単一ドナーからの試験された細胞の生物的状態を反映している。この例の基準は同一の個体から得られた処理しないままの細胞から導出される。４乃至６時間に亘ってリポ多糖類（ＬＰＳ）、熱死滅ブドウ球菌（ＨＫＳ）及びフィトヘマグリチニン（ＰＨＡ）にさらされた細胞につき較正されたプロファイルが示される。図１３ｃは血液サンプル９９１０２８に関してＬＰＳ刺激された血液サンプル９９１１１６の直接比較を示す。即ち、９９１０２８は較正器又は基準プロファイルデータセットとして用いられる。１０／２８／９９で測定

されたメッセンジャーＲＮＡレベルは、１１／１６／９９で測定されたメッセンジャーＲＮＡのレベルと比較するために用いられた。ＲＮＡレベルの完全な同一性は、単位１としての平坦ラインによって表されるであろう。これらのデータは、基準遺伝子発現に関して伝達子ＲＮＡレベルで８倍程度の差（ｃ－ｊｕｎ）があり得ることを明示している。しかし、測定された大抵の遺伝子につき、一日に測定されたメッセンジャーＲＮＡのレベルは異なった日に測定されたものの２乃至３倍以内である。１３ｄ）は、細胞がＬＰＳで刺激されなかったことを除けば１３ｃ）と同様である。

【０１２１】

較正された精密なプロファイルが適切に誘導されている多くの遺伝子座に亘る遺伝子発現の測定から決定される場合全体を通して、当該図は単一の個体内のプロファイルに関して比較的低い変動性を立証する。同図は、１）クラス特有の効果（例えば、ＴＮＦアルファ、ＩＬ－１アルファおよびＩＬ－ベータの好炎症性遺伝子座への効果によって決定されるような概して炎症性の効果）、２）薬剤特有の効果（同一遺伝子座、例えば、ＩＬ－２における各薬剤間の量的差）および３）被験体集団、ＴＫ（図１３ｃ）への再現可能な、従って予想可能な効果を例示する。

【０１２２】

例５．単一薬剤の細胞集団への効果の類似性および相違は集団の生物的状態で異なる。

【０１２３】

生体外遺伝子発現分析は、例えばヘパリンナトリウムを抗凝血剤として血液を真空容器管内へ引き込んで被験体の血液を得ることによって行なわれる。最終濃度が１０ミクロモルの３－メチル－プレドニゾロンのような抗炎症剤がポリプロピレン管内の血液に加えられ、５％CO_2内で３０分間３７℃で培養された。３０分後１０ｎｇ／ｍＬのＬＰＳ又は１：１００希釈の熱死滅ブドウ球菌（ＨＫＳ）が薬剤処理された全血に加えられた。別段の指示がない限り培養は５％CO_2内で６時間に渡り３７℃で継続された。赤血球がＲＢＣリーシス溶液（Ａｍｂｉｏｎ）内で溶解され、残余の細胞がＡｍｂｉｏｎ ＲＮＡｑｕｅｏｕｓ－Ｂｌｏｏｄ ｍｏｄｕｌｅ（ｃａｔａｌｏｇ ＃ １９１３） に従って溶解された。ＲＮＡはＡｍｂｉｏｎ溶離溶液内で溶離された。ＲＮＡは、１ＸＤＮアーゼ緩衝液内において３７℃で３０分間１単位のＤＮアーゼＩ（Ａｍｂｉｏｎ＃２２２２）でＤＮアーゼ化処理された。第１鎖合成はＰｅｒｋｉｎ－Ｅｌｍｅｒ ＴａｑＭａｎ Ｒｅｖｅｒｓｅ Ｔｒａｎｓｃｒｉｐｔａｓｅ ｋｉｔ ｗｉｔｈ ＭｕｌｔｉＳｃｒｉｂｅ ｒｅｖｅｒｓｅ ｔｒａｎｓｃｒｉｐｔａｓｅ（ｃａｔａｌｏｇ ＃ Ｎ８０８－０２３４）を用いて行なわれた。ＲＴ反応の品質検査は、ＰＥ Ｂｉｏｓｙｓｔｅｍｓ（Ｐａｒｔ ＃４３１０８９３Ｅ）からの予備開発された分析試薬（ＰＤＡＲ）１８Ｓ ｒＲＮＡを用いてＴａｑｍａｎ ＰＣＲ化学作用で行なわれた。Ｓｏｕｒｃｅ Ｐｒｅｃｉｓｉｏｎ Ｐｒｏｆｉｌｅｓ のＰＣＲアッセイは、ＰＥ Ｂｉｏｓｙｓｔｅｍｓ ７７００での４複製の６乃至２４遺伝子につき行われた。ＰＣＲアッセイはＰＤＡＲ製品で概説された仕様に従って行われた。興味のある遺伝子の相対的定量は、ＰＥ製品Ｕｓｅｒ Ｂｕｌｌｅｔｉｎ２（１９９７）に記載されかつＧＡＰＤＨの代わりに１８Ｓを用いてＨｙｒａｙａｍａ他（Ｂｌｏｏｄ ９２，１９９８：４６－５２）により詳説されている１８Ｓ ｒＲＮＡ発現に対して較正された。

【０１２４】

ｍＲＮＡの相対的定量は、１８Ｓおよび興味のある遺伝子間の閾値サイクルの差によって測定された。次いで、このデルタC_Tは、棒グラフ（図１４）に表された「折りたたみ誘導」を測定するために規格化状態、即ち、処理前の被験体又は生体外アッセイでの薬剤のない刺激のいずれかに対して比較された。

【０１２５】

例６．生体内および生体外サンプルは比較できる徴候プロファイルを与える。

【０１２６】

図１５は、コルチコステロイド、デキサメタゾンの標準投与量で三日間の期間にわたって処理されて来た２被験体（被験体１および被験体２）につき較正されたプロファイルデー

タセットを示す。各被験体からの血液は７２時間後に入手され、パネル構成成分に対応するＲＮＡの量の定量測定が行われた。各被験体に対する較正されたプロファイルデータセットは大抵の遺伝子座につき類似したが、例えば、Ｉｌ－２、Ｉｌ－１０、Ｉｌ－６およびＧＭ－ＣＳＦに対しては幾つかの顕著な差も同様に検出された。処理前のサンプル１からの血液の生体外サンプルにつきコルチコステロイドと比較するために１組の較正されたプロファイルデータも同様に示され、そこでは生体外サンプルは被験体のプラスマ（血漿）レベルとなるべく計算された試験管内コルチコステロイドの等量にさらされる。生体内サンプルと比較され場合生体外サンプルに対して計算されたプロファイルデータセットの類似性は、当該化合物の生体内作用を予測する試験管内分析に対し支持を与える。本発明者等は、感染性物質、特にバクテリア又はウイルス性病原体で感染された生体内および生体外サンプル間の類似の比較できる影響を観察してきた。従って本発明者等は生体外サンプルが単一化合物又は複数の化合物の被験体に対する影響を決定する有効な方法を与えると結論づける。そこでは被験体の生物的状態に対して薬剤の選択を最適化するために複数の化合物は組合わされるか、並行するか又は順次的に用いられ得る。

【０１２７】

例７．異なった５ドナー被験体に対する立証済抗炎症性を用いた試験管内応答再現性の立証

図１８ａ乃至１８ｅの比較および分析は、異なった５ドナー（各図は独特なドナーを表す）に対する立証済の抗炎症性を用いた刺激および試験管内処理効果の一貫性を立証する。既知および試験済み刺激の使用は、予測可能な生体内反応と相関され得る高度に再現され得る試験管内遺伝子反応に帰着する。図１８ａ－１８ｅは、血液サンプルがとられる５ドナーの分析結果を与える。血液サンプルは、０．１μＭ乃至５μＭ、特に０．１μＭ、０．３μＭ、１μＭ、３μＭおよび５μＭの各種の濃度において４時間期間に亘って治療剤にさらされた。薬剤の異なった濃度は、定性的に次のものとは異なった各濃度における炎症性パネルに対する較正されたプロファイルデータセットに帰着した。図１８ａはドナー１に対応し、図１８ｂはドナー２に対応し、図１８ｃはドナー３に対応し、図１８ｄはドナー４に対応し、また図１８ｅはドナー５に対応する。各個々のものは他のものと異なると共に同様に異なった濃度に対して変動するプロファイルを与えた。図のこの組は較正されたプロファイルデータセットによって得られる高レベル情報を例示する。

【０１２８】

例８．一組の較正されたプロファイルデータは、化合物の複雑な混合物に対して徴候プロファイルを与え得る。

【０１２９】

図２１は、３つの異なった抗炎症性ハーブ（薬草）の炎症性パネル（ＴＮＦＡ－ａ，Ｉｌ－Ｉｂ，ＩＣＡＭ，Ｉｌ－８，Ｉｌ－１０，Ｉｌ－１２ｐ４０，ＩＣＥ，ｃｏｘ－２，ｃｏｘ－１，ｍｍｐ－３）、細胞成長および分化パネル（ｃ－ｆｏｓ，ｃ－ｊｕｎ，ＳＴＡＴ３）、毒性パネル（ＳＯＤ－１，ＴＡＣＥ，ＧＲ，ＨＳＰ７０，ＧＳＴ，ｃ－ｆｏｓ，ｃ－Ｊｕｎ，ＩＮＯＳ）および肝臓代謝パネル（ＩＮＯＳ，ｃｙｐ－ａ，ｕ－ｐａ）の構成成分を含む構成成分のパネルへの影響を例示する。

【０１３０】

図２１で分析された細胞は被験体からの血液の等分部分であり、それらはリポ多糖類およびエキナシア（ＳＰＭ９９１０２１４）、ウサギキク（ＳＰＭ９９１００７６）、シベリアヤクヨウニンジン（ＳＭＰ９９１００７４）に生体外でさらされ、機能食品の各々が２００μｇ／ｍｌの同一濃度で血液サンプルに加えられる。

【０１３１】

基準は機能食品がないリポ多糖類を有する細胞サンプルである。各機能食品（複合混合物から形成された）は、ちょうど単一化合物薬剤の抗炎症剤が持ったように特性徴候プロファイルを有する。同徴候プロファイルは、ハーブを同定するのに用い得ると同時にその特性および単一被験体又は各被験体の平均集団に対するその効率に関する情報を与えるグラフ形式で与えられ得る。

【０１３２】
例９．較正されたプロファイルデータセットを用いたエキナシアの銘柄の品質管理分析
図２４は、４つの異なったエキナシアの市販銘柄に対する較正されたプロファイルデータセットのグラフ式表現を示す。銘柄は「炎症性パネル」を用いる。予期されるように、ＳＰＭ００７およびＳＰＭ００３は該徴候に真正のエキナシアと類似の較正されたプロファイルを与えた。サンプルＳＰＭ０１０およびＳＰＭ０１６は、図１４に記載されたシステムを用いて試験されたときエキナシアのラベルで売られていたが、リポ多糖類単独で得られるプロファイルと実質的に類似の較正された徴候プロファイルに帰着した。　エキナシアサンプルＳＰＭ０１０およびＳＰＭ０１６は生物的に高度の内毒素活性レベルに高められていることが発見されたが、ＳＰ００７およびＳＰ００３のＬＰＳレベルは検出できなかった。効力および作用モードを試験するために設計された、例えば、炎症性パネルから得られる活性エキナシアに関して記憶された徴候プロファイルは、エキナシアの新しいバッチの評価、現存又は新しいエキナシア銘柄の分化、エキナシアのような複合化合物と異なるか又は類似の活性を有する化合物の分離開発の管理を可能にするか又は新しいか若しくは先に市販された化合物の生産、分析および販売の品質保証の開発に用いられ得る。上記例では、エキナシア　ＳＰ０１０およびＳＰ０１６の２つの銘柄は真正エキナシアの特性である較正されたプロファイルに帰着する。
【０１３３】
例１０．指標細胞ラインを用いる３ハーブ調製の比較
図２５ａ）－ｃ）は、被験体からの血液サンプルよりはむしろ指標細胞ライン（ＴＨＰ－１）に関して３つのハーブ調製用に較正されたプロファイルデータセットを与える。図２５ａ）では、基準はハーブのないＴＨＰ－１細胞に対するプロファイルデータセットであり、一方ヒストグラムは、２５０μｇ／ｍｌでの同一ハーブの３つの異なった製造源からの同一ハーブに対する較正されたプロファイルデータセットを表す。遺伝子発現結果は対数スケールで示される。図１４の観察と同様に、特定のパネルｅｇを用いて、これらは異なった源から得られた同様にラベルづけられた化合物が較正されたプロファイルの明白かつ定量可能な差を有することを立証する。遺伝子製品の発現に関する情報を得るために設計された炎症パネルは炎症および感染に関連している。これは特定の目的に用いられるとき化合物は多分異なった効力を有することを示唆する。
【０１３４】
図２５ｂ）は、ＴＨＰ－１の指標細胞ラインを用いて３つの濃度での単一ハーブの較正されたプロファイルを比較している。基準プロファイルデータセットは、処理されていないＴＨＰ－１細胞である。同データの分析は、指標細胞ラインの濃度依存反応を示唆し、本明細書では立証されているが、それは被験体の同様な反応を示し得る。
【０１３５】
図２５ｃ）は、同一濃度で用いられかつ指標細胞集団としてＴＨＰ－１細胞ラインを用いて構成成分のパネルに対して試験される４つの商用エキナシア銘柄が比較される。較正されたプロファイルの差で示される示差表現は複合化合物の直接比較を可能にする。例えば、較正されたプロファイル上の差の分析は、化合物の分離および開発、市場での製品区分（差別化）を管理するために用いられ得るか又はある種の類似化合物から特定の生物的状態に適する単一化合物を個別的に選択することを管理するために消費者又は保健専門家によって用いられる。
【０１３６】
本発明の各種の実施形態が開示されたが、本発明の真の範囲から逸脱することなく本発明の幾つかの利点を達成する各種の変更および改変がなされ得ることは当業者にとって明らかである。これらおよび他の自明な改変は添付の請求の範囲によって保護されることが意図されている。
【図面の簡単な説明】
本発明の前述の特徴は、添付の図面に言及した以下の説明を参照して容易に理解されるだろう。

【図１】
図１は、分子薬理学、及び毒物学、臨床試験で入手されたデータからの情報の流れと、該データの個性化された薬へ適用のための使用を示す概略図である。
【図２】
図２は、初期リードから有望な薬の候補への新しい化合物の薬発見への道を示す概略図である。較正されたプロファイルデータのセットが前臨床段階で示されるが、遺伝子発現データ入手でき、示された任意の段階で有用である。ＩＮＤは、調査新薬（ｉｎｖｅｓｔｉｇａｔｉｖｅ　ｎｅｗ　ｄｒｕｇ）といい、調整検査の初期の段階をいう。
【図３】
図３は、本発明のいくつかの態様による製品候補の毒性と効能を迅速に評価するための較正されたプロファイルデータセットを作る生体外及び生体内の臨床試験計画表の比較を示す概略図である。
【図４】
図４は、本発明のいくつかの態様による、前臨床及び臨床研究の指針として遺伝子発現プロファイルの適用を示す概略図である。
【図５】
図５は、本発明の態様による、刺激のある場合とない場合でのプロファイルデータを得る方法を示す概略図である。
【図６】
図６は、本発明の態様による、複数の被験体に関連したプロファイルデータのライブラリーの創設を示す概略図である。
【図７】
図７は、本発明の態様による、プロファイルデータ記録の構造を例示する概略図である。
【図８】
図８は、本発明の態様による、図７で示された型のデータ記録用のデータ入力スクリーン及びデータ記録が収納され得る典型的なコンテクストを例示する概略図である。
【図９】
図９は、ネットワーク上で遠くからアクセスできるデータベースからのデータを用いて、未加工又は較正された型のプロファイルデータが評価される、本発明の態様を示す。
【図１０】
図１０は、遺伝子発現プロファイリング（ａ）を利用する、フェーズ２臨床試験の概要を示す。右手のパネル（ｂ）は、既に認可され、販売されている薬剤の効能を比較するため、又はそのような治療の販売に導くため；適切な化合物類の種類の中から、個々の被験体又は集団のための治療の選択に導くために、フェーズＩＶ又は販売前の研究において同じ情報が用いられ得ることを示す。
【図１１】
図１１は、１２の構成成分からなるパネル（各々の構成成分は特有の遺伝子座に対応する）を用いて、全血試料の細胞中のＲＮＡの定量的な遺伝子発現を基にした較正されたプロファイルデータセットを示すヒストグラムの型のグラフによる表現を示す棒グラフである。（ａ）血液試料は熱死滅ブドウ球菌で生体外（試験管内で）刺激され、示されているように、更にＨ７－ＴＰＣＫ、Ｈ９－ＵＴ－７７、又はＨ１６－Ｄｅｘにさらされた。（ｂ）血液試料はリポ多糖（ＬＰＳ）で生体外において刺激され、そして示されているように、化合物Ｈ７－ＴＰＣＫ、Ｈ９－ＵＴ－７７、又はＨ１６－Ｄｅｘに更にさらされた。
【図１２】
図１２は、９の構成成分からなるパネル（各々の構成成分は、指示された遺伝子生成物に符号化する遺伝子座に対応する）を用いて、リポ多糖類（ＬＰＳ）で生体外において刺激された全血の較正されたプロファイルデータセットのグラフによる表現を示す対数軸の棒グラフであり、該血液は抗炎症剤、即ち、メトトリキセート、メクロフェナメート、及びメチルプレドニゾロン、に更にさらされる。基準プロファイルデータセットは、ＬＰＳ刺激された（その他の点では未処理）細胞から得られる。

【図１３】
図１３は、２４の構成要素からなるパネル（各々の構成要素は遺伝子座に対応する）を用いて、細胞の生物学的状態を反映する全血（ａ）９９１１１６及び（ｂ）９９１０２８の２種の異なる試料の較正されたプロファイルデータセットのグラフによる表現を示す対数軸の棒グラフであり、基準プロファイルデータセットは、未処理の細胞から得られる。３つの炎症誘引剤（リポ多糖類、熱死滅ブドウ球菌、及びフィトヘマグルチニン（ｐｈｙｔｏｈｅｍａｇｌｕｔｉｎｉｎ））に６時間さらされた細胞について較正されたデータセットが、各々の試料について比較される。（ｃ）は、基準プロファイルデータセットとして、９９１０２８についてＬＰＳ刺激された９９１１１６の直接比較を示し、（ｄ）は刺激されていない９９１１１６と９９１０２８間の直接比較を示す。

【図１４】
図１４は、２２の構成成分からなるパネル（各々の構成成分は遺伝子座に対応する）を用いて、較正されたプロファイルデータセットのグラフによる表現を示す対数軸の棒グラフであり、基準プロファイルデータセットは、未処理の細胞から得られる。全血は、３つの炎症誘引剤（リポ多糖、熱死滅ブドウ球菌、及びフィトヘマグルチニン）に６時間曝露され、それから、生物学的状態において異なる細胞集団への１つの作用因子の効果の類似点と相違点明らかにするため、１つの抗炎症剤（メチルプレドニゾロン）で処理される。

【図１５】
図１５は、全血に対する較正されたプロファイルデータセットのグラフによる表現を示す対数軸の棒グラフであり、ここで１つの較正されたデータセットが、コルチコステロイド（デキサメタゾン）で生体内において処理された被験体（被験体２）に適用され、第２のデータセットが、生体内処理の前に同じ被験体からの血液試料（その試料は生体外（試験管内）で処理されている）の処理に適用され、そして第３のデータセットが、デキサメタゾンで生体内で処理された第２の被験体（第１の被験体）に適用される。データセットは、同じ作用因子での生体内処理に比べて、血液の生体外（試験管内）処理の再現性と予測可能性を示す。図は、個体間の変異性を反映する異なる被験体からの試料の間で小さな違いも示す。１４の構成成分からなるパネルが提供される。基準プロファイルデータセットは、同源（ｃｏｇｎａｔｅ）の被験体からの未処理の全血から得られる。

【図１６】
図１６は、全血に対する較正されたプロファイルデータセットのグラフによる表現を示す対数軸の棒グラフであり、ここで１つの較正されたデータセットが、（ａ）未活性のプラシボーで３日間、（ｂ）活性プレドニゾロンを１００ｍｇ／日で３日間生体内において処理された２つの被験体に適用される。データセットは、同じ薬剤で処理された異なる被験体からの試料間での多少の違いを示す。データセットは、同じ遺伝子座での応答の類似点、並びに量で表される個体間の違いを示唆する他の座での定量的な違いを示す。８つの構成要素からなるパネルが提供される。基準プロファイルデータセットは未処理の全血から得られる。

【図１７】
図１７は、２４の構成成分（各々の構成成分は独特の遺伝子座に対応する）からなるパネル（炎症パネル）を用いている１９日間の間に１人の被験体から採取された２つの試料に対する、較正された精密なプロファイルデータセットのグラフによる表現を示す対数のｙ軸の棒グラフである。基準プロファイルデータセットは、処理前の被験体から採取された末梢血液に関連する。

【図１８】
図１８（ａ－ｅ）は、血液試料が採取された５つの被験体の各々に対する、較正されたプロファイルデータセットのグラフによる表現を示す対数軸の棒グラフである。血液試料の各々は、被験体を処理するのに最適な投与を決めるため、生体外（試験管内）で４時間の間、炎症剤フトヘマグルチニン（ＰＨＡ）、又は治療剤（抗炎症剤）に異なる濃度で（０．１μＭ、０．３μＭ、１μＭ、３μＭ、及び５μＭ）さらされた。６つの構成成分からなるパネルが、６つの遺伝子座に対応して用いられた。基準プロファイルデータセットは

、同源のドナーから得られる未処理試料であった。

【図１９】

図１９は、２４の構成成分を有するパネルを用いる、異なる生物学的条件を有する３人の異なる被験体に対する、較正されたプロファイルデータセットのグラフによる表現を示す対数軸の棒グラフである。プロファイルデータセットは、診断上の徴候パネルに対する基準を提供するこれらの条件に応じて変異性を示す。（ａ）は、禁煙者の基準に対する喫煙者の較正されたプロファイルデータセットを示す。（ｂ）は、慢性障害肺疾患の被験体の、この疾患のない被験体の基準に対する較正されたプロファイルデータセットを示す。基準プロファイルデータセットは、これらの状態に関し「正常」な被験体から得られる。

【図２０】

図２０は、個々の応答が同様に処理された集団から区別され得ることを示す。単一の位置（ＧＳＴ－Ｐ）に関して、実験群（ｎ＝５の動物）に対する一匹の動物の応答の比較が提供される。基準データセットは、群の平均である。この動物は、調査の最初の２日での毎日の集団平均から著しく異なっているが、アセタミノフェンで処理した後時間とともに構成要素平均により類似したことを図は示す。

【図２１】

図２１は、生体外で２００μｇ／ｍｌの濃度のＬＰＳ又はＬＰＳと３種の抗炎症ハーブ（エキナシア、ウサギキク、シベリアヤクヨウニンジン）の１つで処理された血液試料に対する、較正されたプロファイルデータセットのグラフによる表現を示す対数軸の棒グラフである。２４の構成成分からなるパネルが用いられる。基準プロファイルデータセットが、ハーブ処理されていないＬＰＳ刺激された細胞から得られる。図は、栄養物のような複合化合物（その生物学的効果は１以上の活性の合計である）の全体の効果を調べるために較正された精密なプロファイルの使用の有効性を例示する。この場合、ハーブの各々は免疫刺激剤として消費されるが、較正された精密なプロファイルは、免疫刺激性及び抗炎症効果両方の混合を示す独特なパターンを示す。

【図２２】

図２２は、生体外でＬＰＳ、又はＬＰＳとメチルプレドニゾロン、又はＬＰＳとウサギキクで処理された血液試料に対する、較正されたプロファイルデータセットのグラフによる表現を示す対数軸の棒グラフである。基準データセットは、ＬＰＳ処理された血液試料である。

【図２３】

図２３は、２２の構成成分からなるパネルを用いて、生体外で３つの異なる濃度でＬＰＳ、又はＬＰＳとウサギキクで処理されたＴＨＰ－１細胞の試料に対する、較正されたプロファイルデータセットのグラフによる表現を示す対数軸の棒グラフである。基準プロファイルデータセットは、未処理のＴＨＰ－１細胞である。図は、較正されたプロファイルにわたる遺伝子発現に関する濃度応答を例示する。

【図２４】

図２４は、８の構成成分からなるパネルを用いて、エキナシアの４つの異なる市販銘柄で生体外で処理されたＴＨＰ－１細胞の試料に対する、較正されたプロファイルデータセットのグラフによる表現を示す対数軸の棒グラフである。基準プロファイルデータセットは、未処理のＴＨＰ－１細胞である。

【図２５】

図２５は、銘柄又は異なる処方中の相対的な効能を比較するための較正されたプロファイルの使用を例示する。指標単球性細胞ライン（ＴＨＰ－１）に関して異なる製造原料からのハーブの調製に対する較正されたプロファイルデータセットがグラフで示され、基準プロファイルデータセットは、ハーブのないＴＨＰ－１細胞である。（ａ）は２５０（μｇ／ｍｌ）での３種の市販ハーブエキナシアの調製、（ｂ）は異なる濃度（２５０μｇ／ｍｌ、５０μｇ／ｍｌ、及び３－１０μｇ／ｍｌ）での３種の市販のハーブ、（ｃ）は２５０μｇ／ｍｌでの４種の市販エキナシア銘柄である。

【図１】

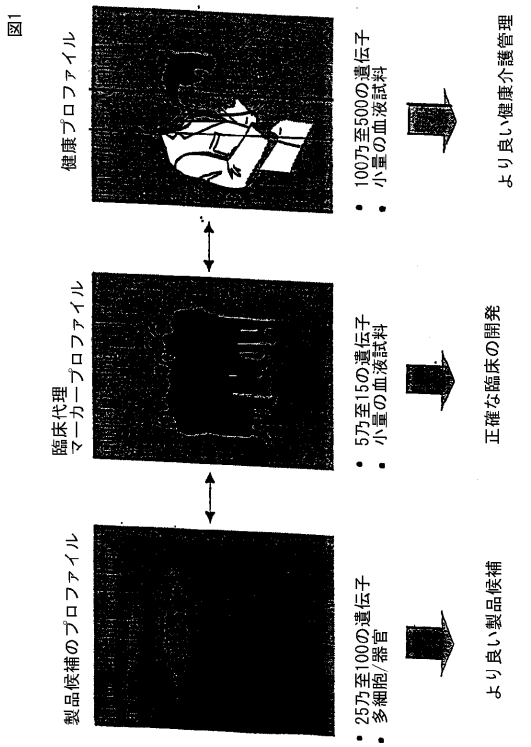


図1
製品候補のプロファイル
臨床代理
マーカープロファイル
健康プロファイル
• 25乃至100の遺伝子
• 多細胞/器官
• 5乃至15の遺伝子
• 小量の血液試料
• 100乃至500の遺伝子
• 小量の血液試料
より良い製品候補
正確な臨床の開発
より良い健康介護管理

【図２】

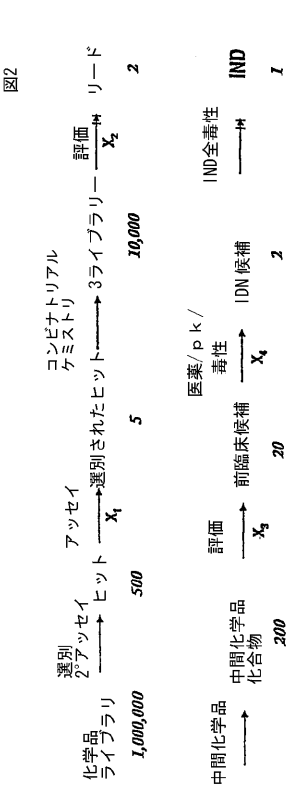


図2
コンビナトリアル
ケミストリ
アッセイ
選別
2°アッセイ
化学品
ライブラリ
ヒット
選別されたヒット
3ライブラリー
評価
リード
1,000,000
500
5
10,000
2
医薬/pk/
毒性
中間化学品
化合物
評価
中間化学品
化合物
前臨床候補
IDN候補
IND全毒性
IND
200
20
2
1
フェーズ1及び2
安全で有効な薬
生理化学分析
試験管内毒性学
遺伝子発現プロファイリング
代理マーカーの生成

【図３】

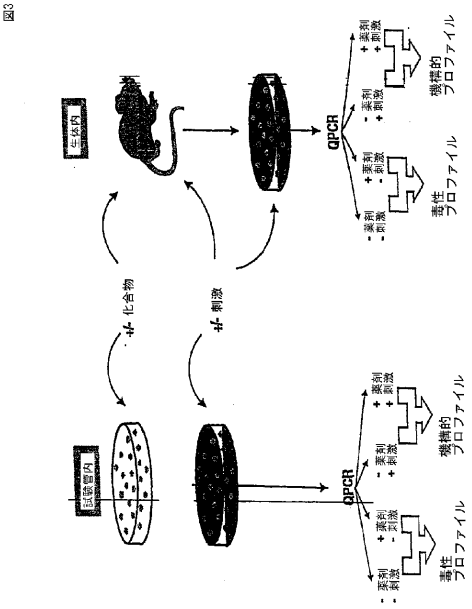


図3
試験管内
生体内
化合物
刺激
QPCR
毒性
プロファイル
機構的
プロファイル

【図４】

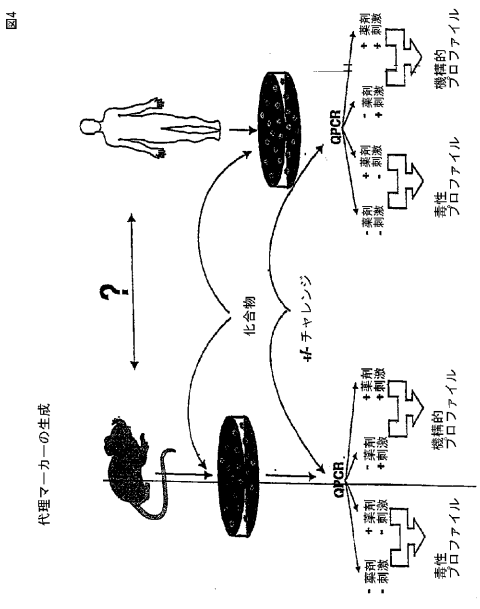
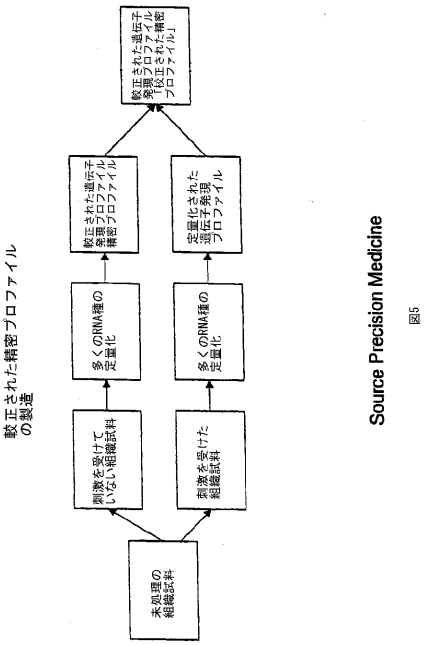


図4
代理マーカーの生成
化合物
チャレンジ
QPCR
毒性
プロファイル
機構的
プロファイル

【図５】


較正された精密プロファイル
の製造
未処理の組織試料
刺激を受けていない組織試料
刺激を受けた組織試料
多くのRNA種の定量化
多くのRNA種の定量化
較正された遺伝子発現プロファイル
定量化された遺伝子発現プロファイル
較正された遺伝子発現プロファイル「較正された精密プロファイル」
Source Precision Medicine
図5

【図６】

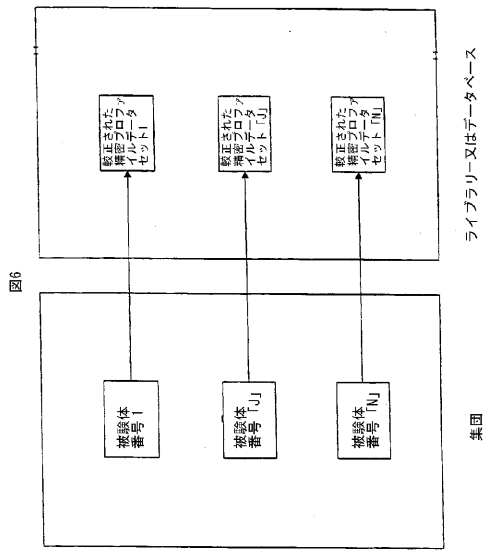


図6
較正された精密プロファイルデータセット1
較正された精密プロファイルデータセット「J」
較正された精密プロファイルデータセット「N」
被験体番号1
被験体番号「J」
被験体番号「N」
集団
ライブラリー又はデータベース

【図７】

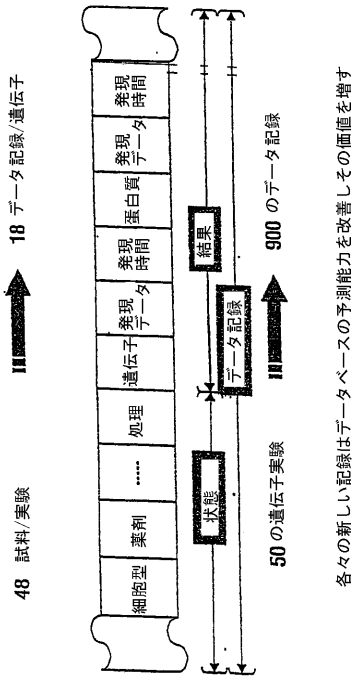


図7
48 試料/実験
18 データ記録/遺伝子
細胞型
薬剤
.....
処理
遺伝子
発現データ
発現時間
蛋白質
発現データ
発現時間
状態
データ記録
結果
50の遺伝子実験
900 のデータ記録
各々の新しい記録はデータベースの予測能力を改善しその価値を増す

【図８】

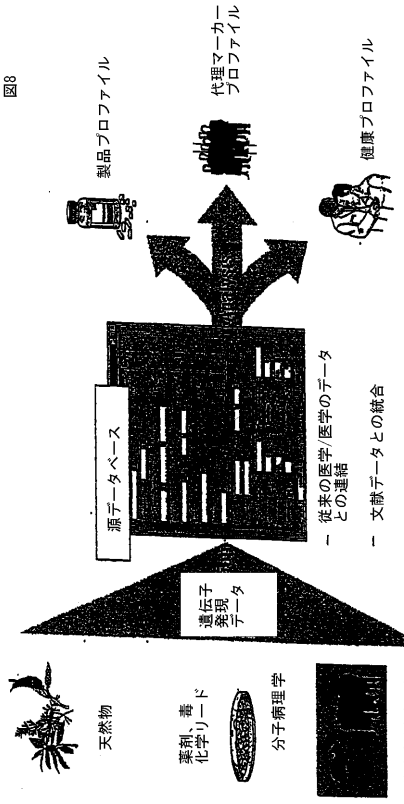


図8
製品プロファイル
代理マーカープロファイル
健康プロファイル
源データベース
遺伝子発現データ
－ 従来の医学/医学のデータとの連結
－ 文献データとの統合
天然物
薬剤、毒
化学リード
分子病理学
前臨床/臨床試料

【図９】

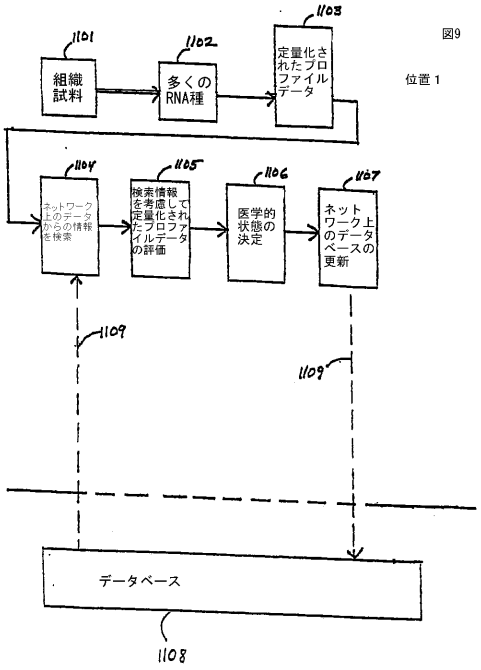


図9

【図１０】

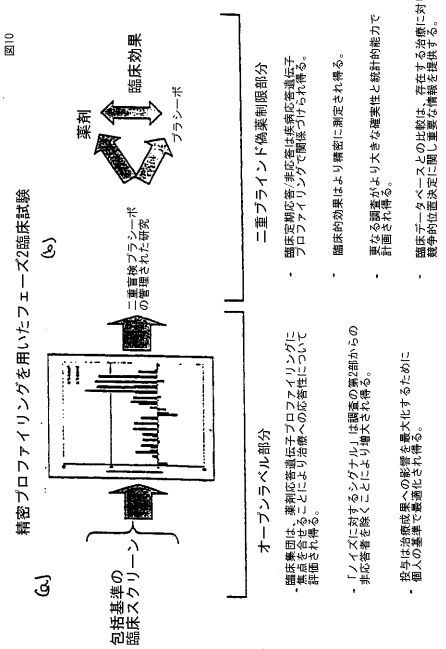


図10

【図１１】

図11（a）

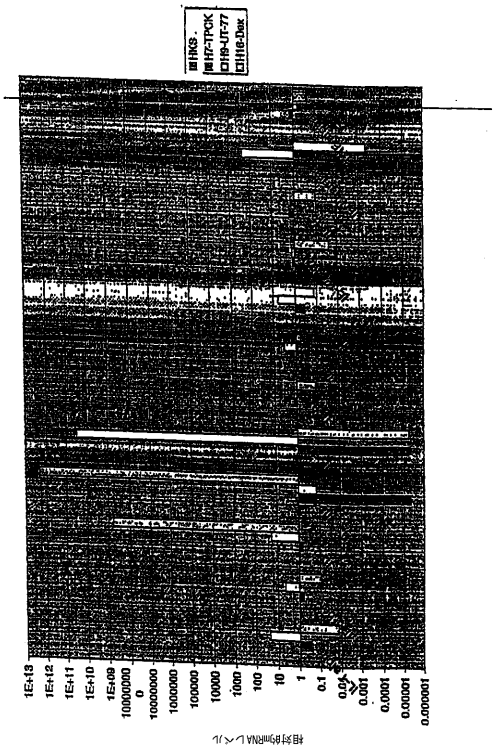
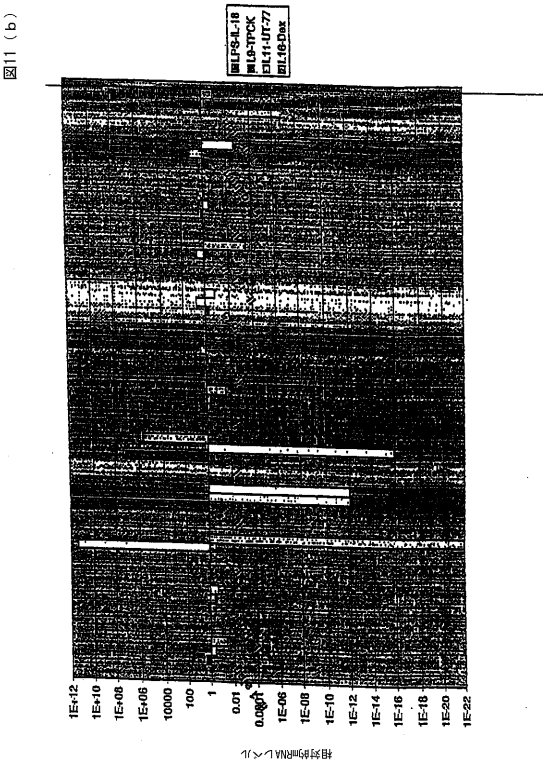


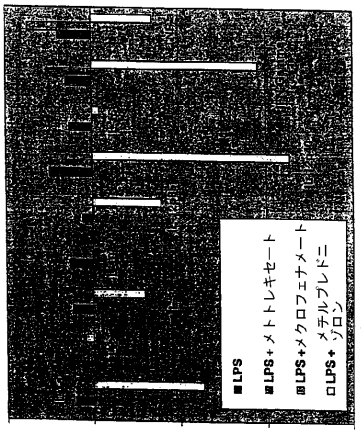
図11（b）



【図１２】

図12

比較薬剤プロファイリングは、異なる作用機構の抗炎症薬中の相違を示す



【図１３】

図13（a）

99116LPS、HKS、PHAのWB6時間の比較刺激

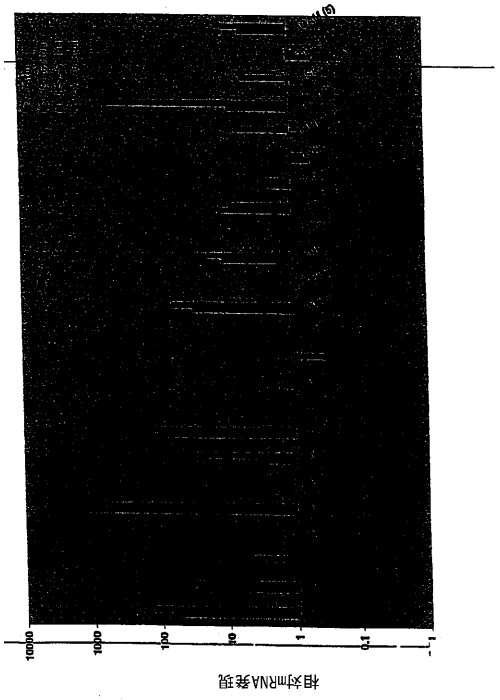


図13（b）

991028LPS、HKS、PHAのWB6時間の比較刺激

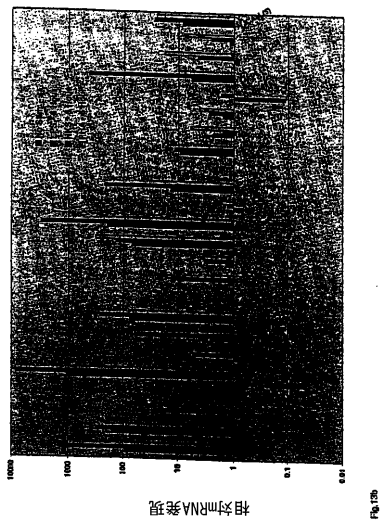


図13（c）

LPS刺激の個々の比較　991028対991116　ドナー：TK

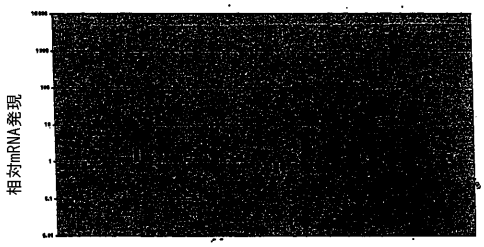
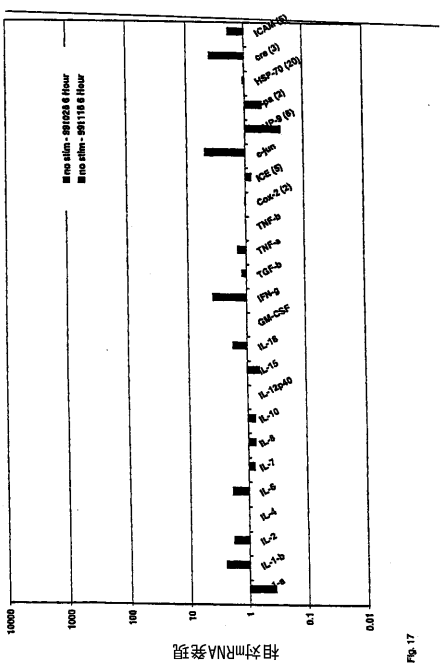
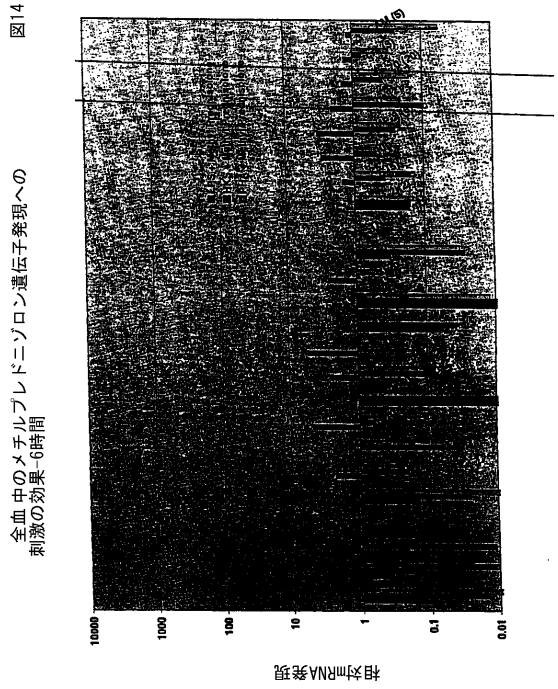


図13（d）

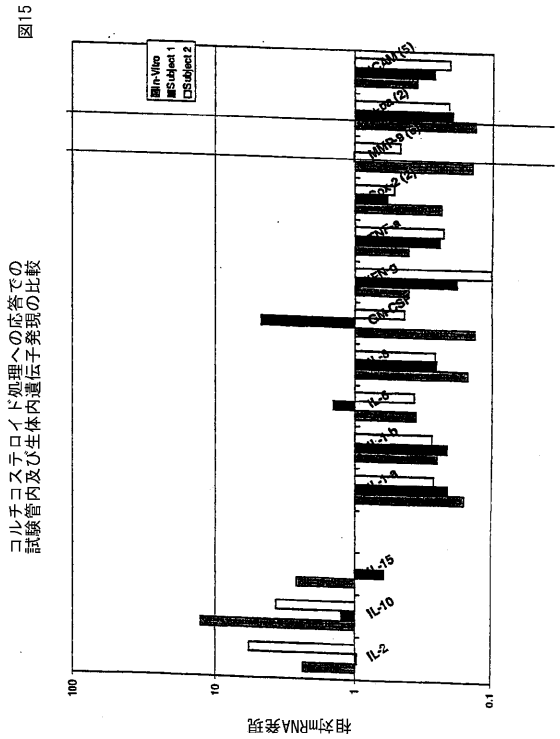
6時間刺激のないドナー試料の個々の比較
991028対991116　ドナー：TK



【図１４】



【図１５】



【図１６】

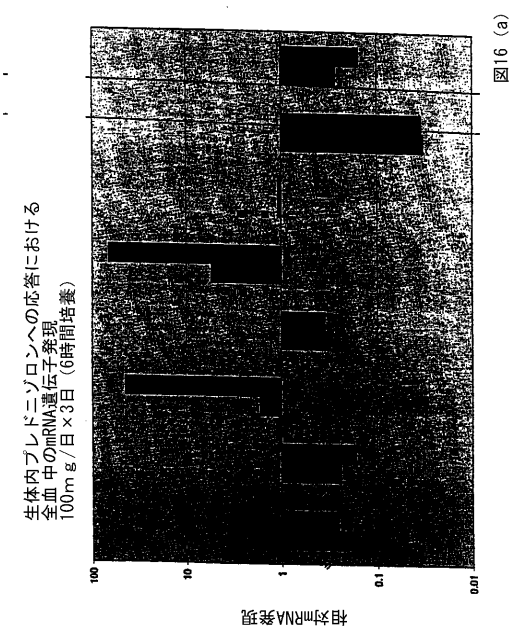


図16（a）

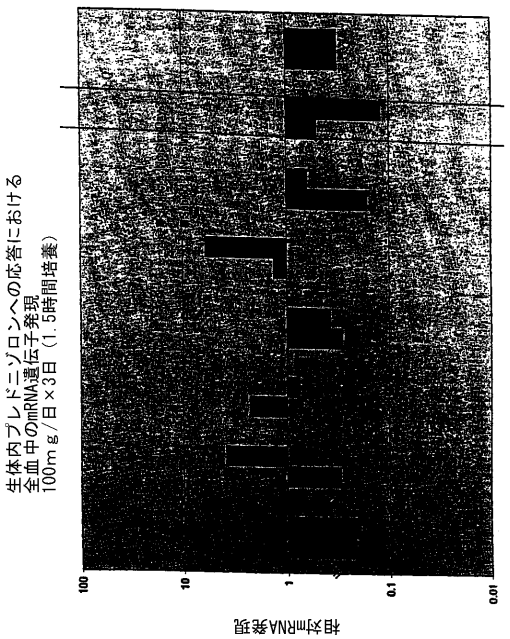


図16（b）

【図１７】

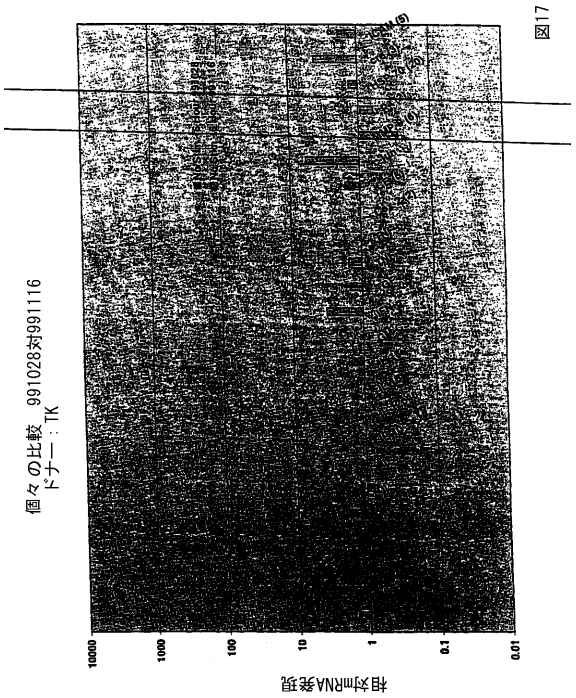


図17

【図１８】

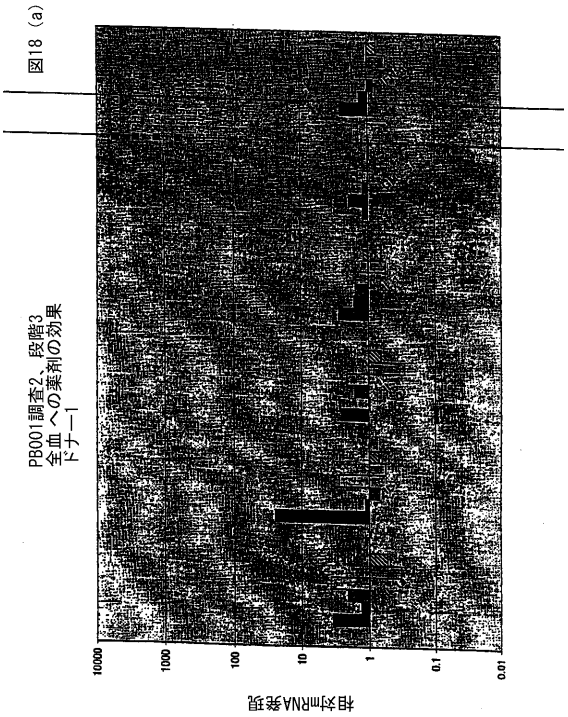


図18（a）

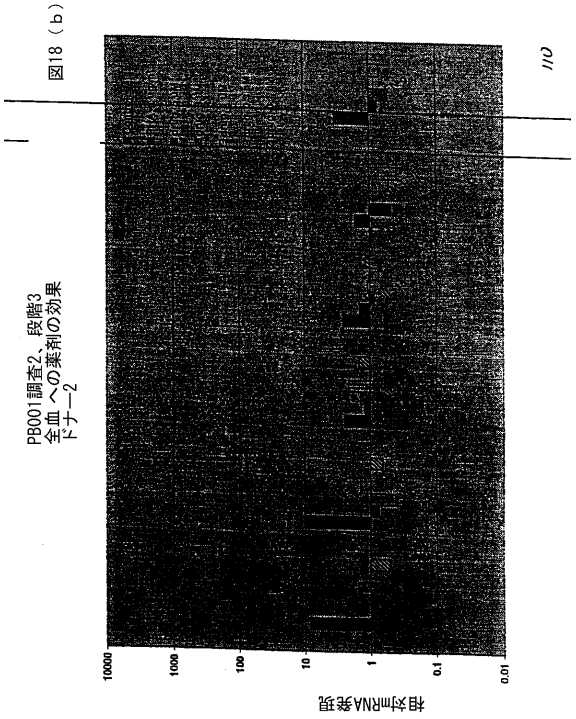


図18（b）

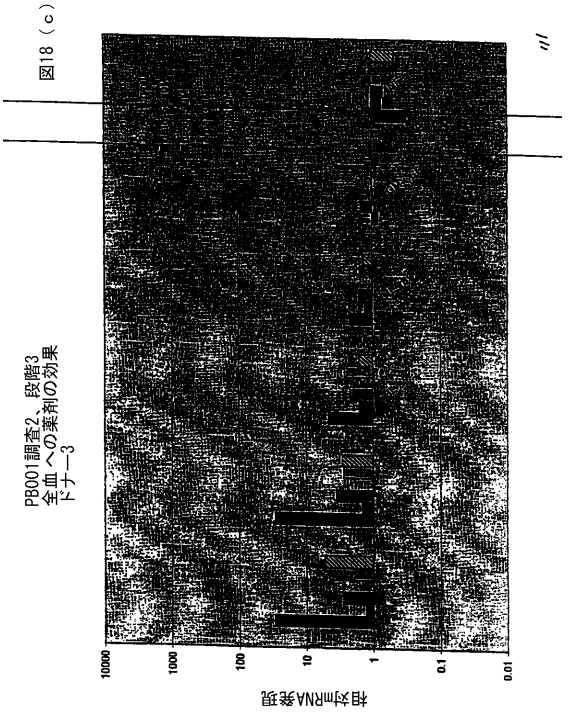


図18（c）

図18（d）

PB001調査2、段階3
PHA刺激された全血 への薬剤の効果
ドナー4

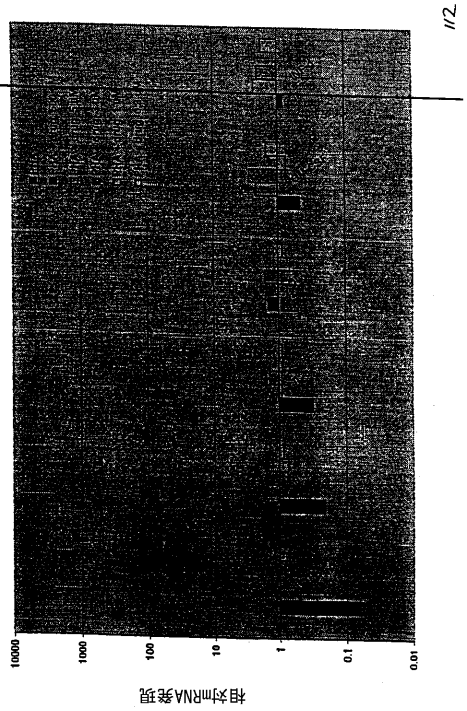
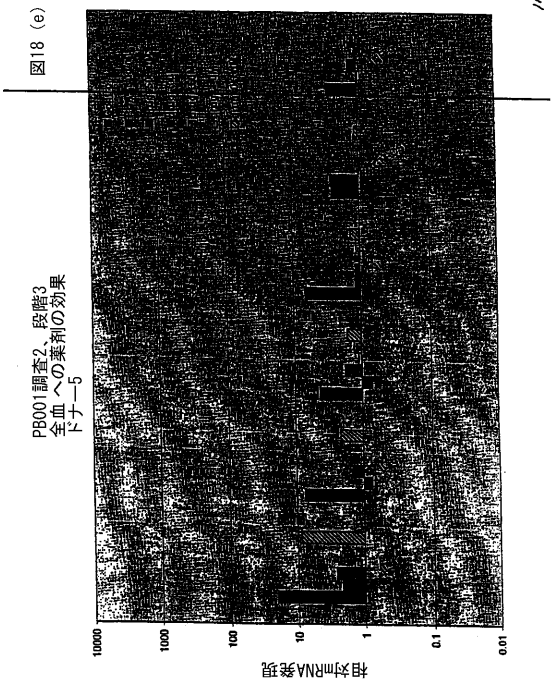


図18（e）

PB001調査2、段階3
全血 への薬剤の効果
ドナー5



【図１９】

図19（a）

喫煙者対正常者

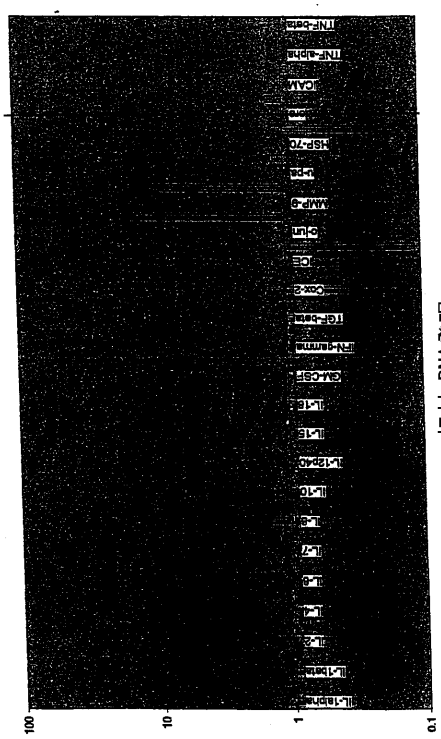


Fig. 24

図19（b）

NAC看者対正常者

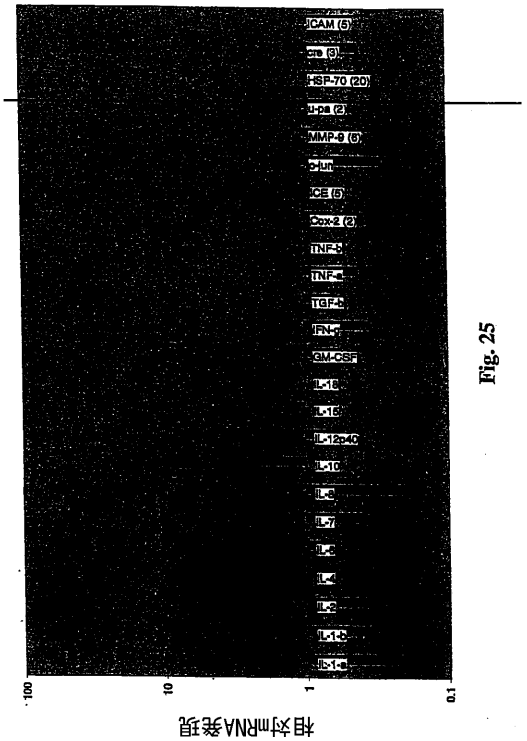
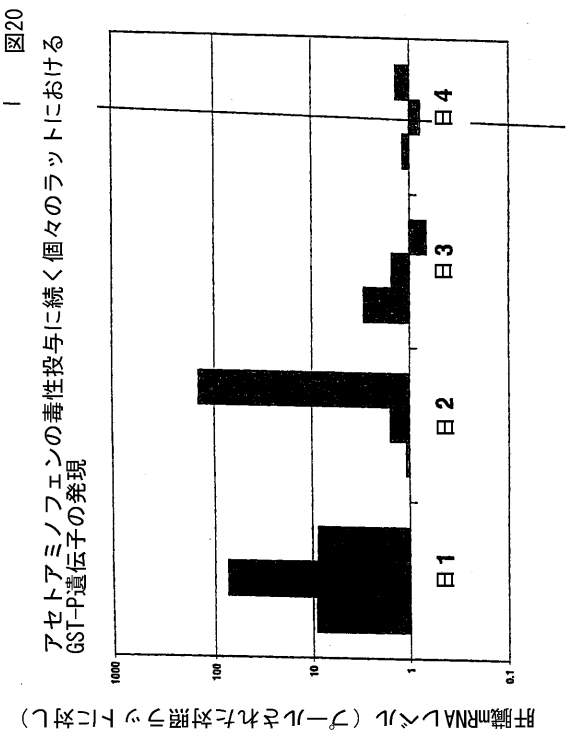
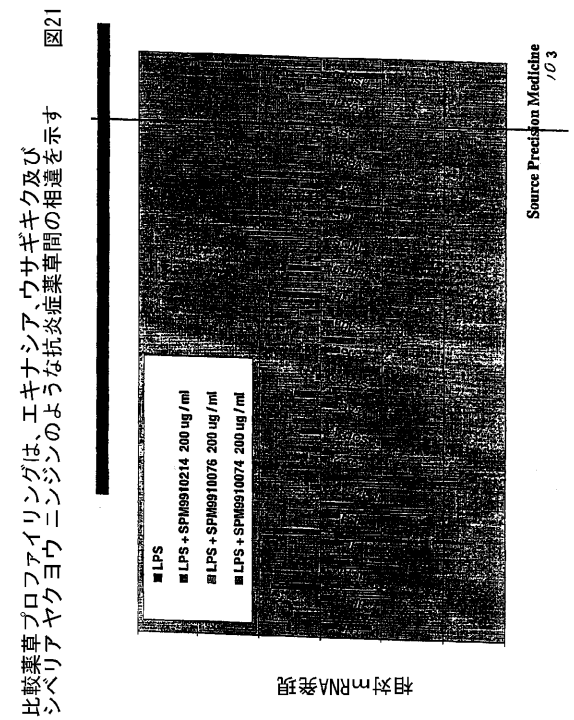


Fig. 25

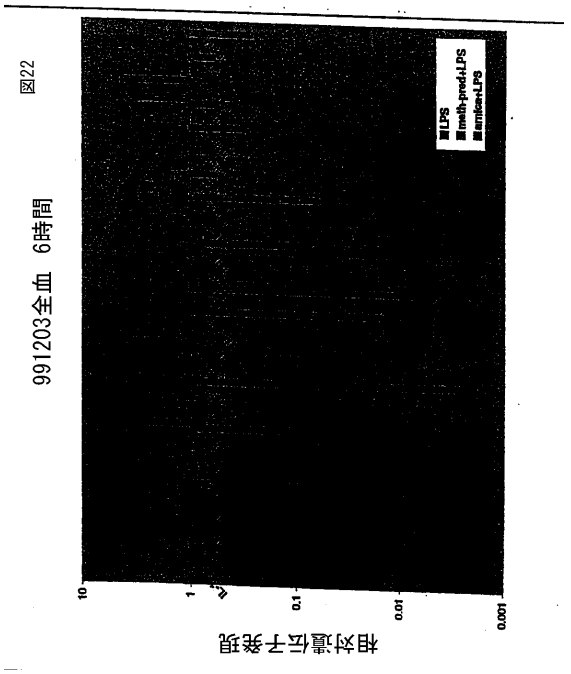
【図２０】



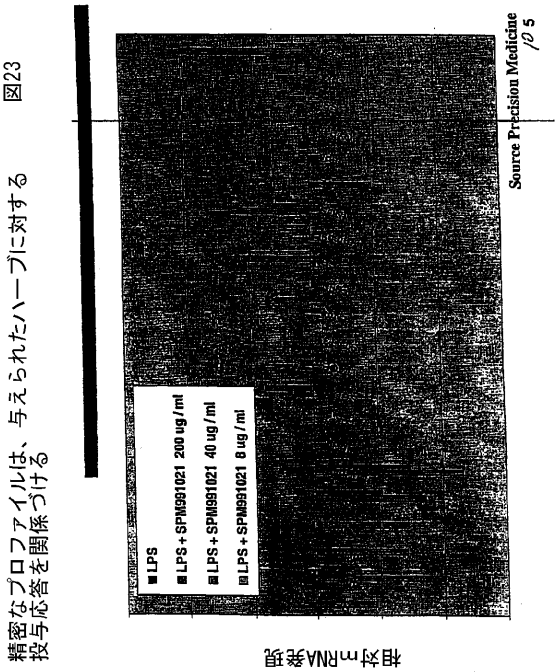
【図２１】



【図２２】



【図２３】



【図２４】

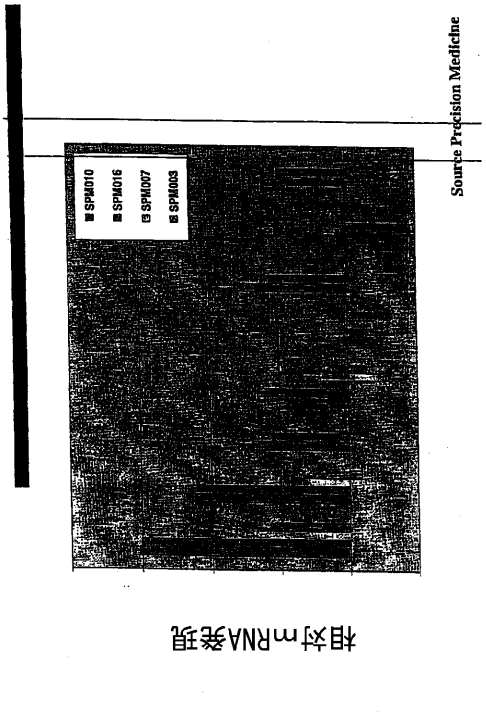


図24
精密なプロファイルは、SPMO10及びSPMO16中で示されるような異なる市販の銘柄中の内毒素との不純物を示す

【図２５】

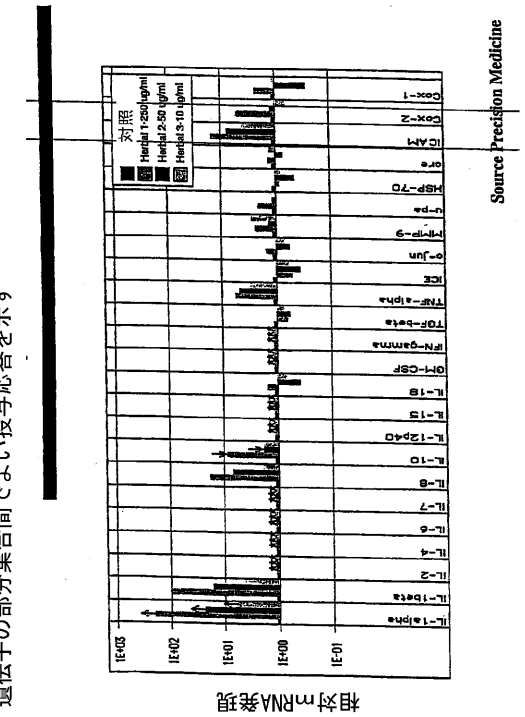


図25（b）
刺激されていないTHP-１細胞の単一のハーブでの処理は、遺伝子の部分集合間でよい投与応答を示す

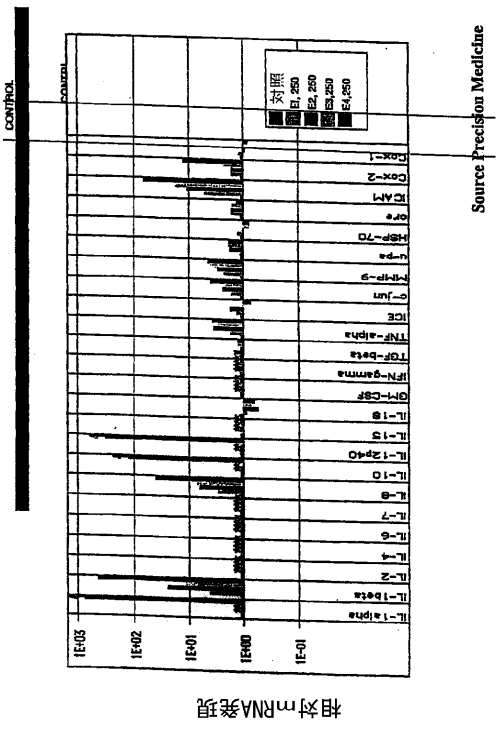


図25（C）
精密なプロファイルは市販のエキナシア（E1-E4）の比較を許容する

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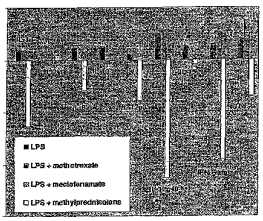
(54) Title: SYSTEMS AND METHODS FOR CHARACTERIZING A BIOLOGICAL CONDITION OR AGENT USING CALIBRATED GENE EXPRESSION PROFILES

Comparative Drug Profiling Shows Differences Among Anti-Inflammatory Drugs with Different Mechanism of Action



(57) Abstract: The invention provides a method, for evaluating a biological condition of a subject, comprising: a. obtaining from the subject a sample having at least one of RNAs and proteins; b. deriving from the sample a first profile data set, the first profile data set including a plurality of members, each member being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of the biological condition; and c. producing a calibrated profile data set for the panel, wherein each member of the calibrated profile data set is a function of a corresponding member of the first profile data set and a corresponding member of a baseline profile data set for the panel, the calibrated profile data set providing a measure of the biological condition of the subject.

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SYSTEMS AND METHODS FOR CHARACTERIZING A BIOLOGICAL CONDITION OR AGENT USING CALIBRATED GENE EXPRESSION PROFILES

Technical Field

A method is provided for identifying reproducible patterns of variation of gene expression that are informative by virtue of the degree of variation observed in a calibrated data set. The variations may be correlated with other non- genetic indications such as clinical indicators (for humans) of a traditional nature but are not required per se to be causative.

Background Art

There has been substantial discussion including congressional hearings concerning medical errors. One source of medical errors include errors with medications. Upwards of 98,000 hospitalized patients annually have been documented to be victims of medication errors (Statement of the American Pharmaceutical Association to the Senate Appropriations Committee Labor, health and Human Services Education Subcommittee Hearing on Medical Errors December 13, 1999). These errors include problems arising from drug interactions for a particular patient taking more than one drug, problems concerning the response of an individual to a particular drug and incorrect medication for a particular condition. Medical errors further arise as a result of misdiagnosis. This may occur as a result of insensitive diagnostic techniques or a wide range of interpersonal variability in the manner in which a clinical state is manifest. At present, there are few tools available for optimizing prognosis, diagnosis and treatment of a medical condition taking into account the particular phenotype and genotype of an individual.

There has been increasing interest in herbal drugs or nutraceuticals. These are often grown in developing countries and undergo little or no quality control. It is frequently the case that one batch of a nutraceutical may be effective, there is no assurance that a second batch will be effective. Moreover, analysis of nutraceuticals is problematic because these drugs are complex mixtures in which little is known with respect to the active agent.

All new therapeutic agents require some form of clinical trials. It is known that a drug for treating tumor that is tested in a clinical trial using standard recruiting techniques for patients, may in fact show only limited efficacy. If the beneficial effect observed in a clinical population is too small, the drug will not receive approval by the Food and Drug Administration for use in the population at large. However, the small beneficial effect observed may in fact be an artifact of the clinical trial design or the clinical endpoint in the population of patients. It would be desirable to have criteria for screening patients as they enter a clinical trial to ensure that the beneficial effect of a drug if it exists may be detected and quantified.

Summary of the Invention

In a first embodiment of the invention there is provided a method, for evaluating a biological condition of a subject, that includes: obtaining from the subject a sample having at least one of RNAs and proteins; deriving from the sample a first profile data set, the first profile data set including a plurality of members, each member being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of the biological condition; and producing a calibrated profile data set for the panel, wherein each member of the calibrated profile data set is a function of a corresponding member of the first profile data set and a corresponding member of a baseline profile data set for the panel, the calibrated profile data set providing a measure of the biological condition of the subject.

In a preferred embodiment, a method is provided for evaluating a biological condition of a subject, that includes obtaining from the subject a first

sample having at least one of fluid, cells and active agents; applying the first sample or a portion thereof to a defined population of indicator cells; obtaining from the indicator cells a second sample containing at least one of RNAs or proteins; deriving from the second sample a first profile data set, the first profile data set including a plurality of members, each member being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of the biological condition; and producing a calibrated profile data set for the panel, wherein each member of the calibrated profile data set is a function of a corresponding member of the first profile data set and a corresponding member of a baseline profile data set for the panel, the calibrated profile data set providing a measure of the biological condition of the subject.

In a preferred embodiment, a method is provided for evaluating a biological condition affected by an agent, the method including: obtaining, from a target population of cells to which the agent has been administered, a sample having at least one of RNAs and proteins; deriving from the sample a first profile data set, the first profile data set including a plurality of members, each member being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of the biological condition; and producing a calibrated profile data set for the panel, wherein each member of the calibrated profile data set is a function of a corresponding member of the first profile data set and a corresponding member of a baseline profile data set for the panel, the calibrated profile data set providing a measure of the biological condition as affected by the agent.

In a preferred embodiment, a method is provided for evaluating the effect on a biological condition by a first agent in relation to the effect by a second agent, including: obtaining, from first and second target populations of cells to which the first and second agents have been respectively administered, first and second samples respectively, each sample having at least one of RNAs and proteins; deriving from the first sample a first profile data set and from the

second sample a second profile data set, the profile data sets each including a plurality of members, each member being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of the biological condition; and producing for the panel a first calibrated profile data set and a second profile data set, wherein (i) each member of the first calibrated profile data set is a function of a corresponding member of the first profile data set and a corresponding member of a first baseline profile data set for the panel, and (ii) each member of the second calibrated profile data set is a function of a corresponding member of the second profile data set and a corresponding member of a second baseline profile data set for the panel, the calibrated profile data sets providing a measure of the effect by the first agent on the biological condition in relation to the effect by the second agent.

In a preferred embodiment, a method of conducting a clinical trial of an agent, is provided, including: causing the blind administration of a selected one of a placebo and the agent to each candidate of a pool of subjects; and using quantitative gene expression to monitor an effect of such administration.

In a preferred embodiment, a digital storage medium is provided on which is stored a computer readable calibrated profile data set, wherein: the calibrated profile data set relates to a sample having at least one of RNAs and proteins derived from a target cell population to which an agent has been administered; the calibrated profile data set includes a first plurality of members, each member being a quantitative measure of a change in an amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of a biological condition as affected by administration of the agent.

In a preferred embodiment, a digital storage medium is provided on which is stored a plurality of records R_i relating to a population of subjects, each record R_i corresponding to a distinct instance P_i of a computer readable profile data set P wherein: each instance P_i of the profile data set P relates to a distinct sample derived from a subject, the sample having at least one of RNAs and

proteins; the profile data P set includes a plurality of members M_j, each member M_j being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of a biological condition; each record R_i includes, for each member M_{ij} of a corresponding distinct instance P_i of the profile data set P, a value corresponding to the value of the member M_{ij}; and each record R_i also includes a reference to a characteristic of the subject relative to the record, the characteristic being at least one of age group, gender, ethnicity, geographic location, diet, medical disorder, clinical indicator, medication, physical activity, body mass, and environmental exposure.

In a preferred embodiment, a digital storage medium is provided on which is stored a large number of computer readable profile data sets, wherein each profile data set relates to a sample derived from a target cell population to which has been administered an agent, the sample having at least one of RNAs and proteins; each profile data set includes a plurality of members, each member being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of a biological condition; and the panel is the same for all profile data sets.

In a preferred embodiment of the invention, a method is provided for evaluating a biological condition of a subject, based on a sample from the subject, the sample having at least one of RNAs and proteins, the method including: deriving from the sample a first instance of a profile data set, the profile data set including a plurality of members, each member being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of the biological condition; and producing a first instance of a calibrated profile data set for the panel, wherein each member of an instance of the calibrated profile data set is a function of a corresponding member of an instance of the profile data set and a corresponding member of an instance of a baseline profile data set for the panel, the calibrated profile data set providing a measure of the biological

condition of the subject; accessing data in a condition database, the condition database having a plurality of records relating to a population of subjects, each record corresponding to a distinct instance of the calibrated profile data set; and evaluating the first instance of the calibrated profile data set in relation to data in the condition database.

In a preferred embodiment of the invention, a method is provided of displaying quantitative gene expression analysis data associated with measurement of a biological condition, the method including: identifying a first profile data set pertinent to the gene expression analysis data, the first profile data set including a plurality of members, each member being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of the biological condition; producing a calibrated profile data set for the panel, wherein each member of the calibrated profile data set is a function of a corresponding member of the first profile data set and a corresponding member of a baseline profile data set for the panel, the calibrated profile data set providing a measure of the biological condition of the subject; and displaying the calibrated profile data set in a graphical format.

A preferred embodiment is directed to a descriptive record of a change in a biological condition, that includes: a first set of numerical gene expression values for a panel of gene loci, each value in the set corresponding to a single gene locus in a panel of gene loci, the set of values forming a profile data set for a population of cells subjected to a first biological condition; a second set of numerical gene expression values for the panel of gene loci, each value in the set corresponding to a single gene locus, the set of values forming a baseline profile data set for a second population of cells subjected to a second biological condition, the second set of values optionally being an average for multiple gene expression values from multiple populations of cells for each locus in the panel; and a third set of numbers corresponding to the ratio of the first set of values and the second set of values with respect to each gene locus in the panel, the third set being a calibrated profile data set; the profile data set and the calibrated

profile data set being descriptive of the first biological condition with respect to the second biological condition.

In a preferred embodiment, a method for diagnosing a biological condition of a subject is provided that includes: obtaining a sample from a subject; subjecting a population of cells to the sample and determining the presence of a first biological condition with respect to a second biological condition according to any of the above claims.

In a preferred embodiment, a method is provided for diagnosing a susceptibility for a biological condition in a subject, that includes obtaining a sample from the subject; creating a descriptive record, according to the above, wherein the baseline set of values is an average of second values contained in a library of descriptive records for the second biological condition; the library containing a plurality of descriptive records grouped according to a predetermined biological condition; comparing the calibrated profile data set of the subject with the library of calibrated profile data sets and diagnosing the susceptibility of the subject.

In a preferred embodiment, a method is provided for monitoring the progress of a biological condition, including: creating a plurality of descriptive records, according to the above; wherein each set of first values is determined at preselected time intervals with respect to the first record; comparing each calibrated profile data set with a library of calibrated profile data sets, the plurality of calibrated profile data sets being grouped according to a predetermined biological condition; and determining the progress of the biological condition with respect to gene expression.

In a preferred embodiment, a method is provided for establishing the biological activity of a composition, including: selecting a population of cells; subjecting the cells to the composition; and determining the record according to the above description using a standardized baseline profile data set for the biological condition.

In a preferred embodiment, a method is provided for determining which therapeutic agent from a choice of a plurality of therapeutic agents to administer

to a subject so as to change a biological condition in a subject from a first biological condition to a second biological condition; including: subjecting a sample from the subject to each of a plurality of therapeutic agents; determining a descriptive record for each of the samples according to any of the above described methods, comparing each of the calibrated profile data sets to a library of calibrated profile data sets, the library of calibrated data sets being grouped according to a predetermined biological condition; and determining which of the therapeutic agents is capable of changing the first biological condition in the subject to the second biological condition in the subject.

In a preferred embodiment, a method is provided for characterizing the biological effectiveness of a single batch of a composition produced by a manufacturing process, comprising: providing a fingerprint or signature profile according to any of the above methods; and labeling the batch of the composition by placing the fingerprint (signature profile) on each container in the batch.

In a preferred embodiment, a method is provided for accessing biological information on a digital storage medium as described above, including: making the information available to a user.

In a preferred embodiment, a method is provided for consumer evaluation of a product , wherein the consumer evaluation is dependent on a signature profile, including: identifying the product using the signature profile.

In a preferred embodiment, a computer program product is provided for evaluating a biological condition of a subject or for evaluating a biological condition resulting from the use of an agent, including a computer usable medium having computer readable program code thereon, the computer program code; including: a program code for classifying a sample from the subject or the agent for an identifiable record; a program code for deriving a first data set, the first profile data set including a plurality of members, each member being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of the biological condition; the profile data set being stored in the record; and a program code for optionally producing a

calibrated profile data set for the panel, for storage in the record, each member of the calibrated profile data set being a function of a corresponding member of the first profile data set and a corresponding member of a baseline profile data set for the panel, the calibrated profile data set providing a measure of the biological condition of the subject.

In a preferred embodiment of the invention, a computer system for evaluating a biological condition of a subject or for evaluating a biological condition resulting from the use of an agent is provided, the computer system, including: a classification module for classifying a sample from the subject or the agent in an identifiable record; a derivative module for deriving a first data set, the first profile data set including a plurality of members, each member being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of the biological condition; and a production module for producing a calibrated profile data set for the panel, wherein each member of the calibrated profile data set is a function of a corresponding member of the first profile data set and a corresponding member of a baseline profile data set for the panel, the calibrated profile data set providing a measure of the biological condition of the subject.

In a preferred embodiment, a method is provided for analyzing a patient for a biological condition at a remote site, including: providing a kit for measuring a profile data base for evaluating a biological condition, the kit including reagents for quantitative analysis of RNA or protein for a panel of gene loci; accessing a centralized database containing baseline profile data sets corresponding to the panel; determining the calibrated profile data set for the patient; and analyzing the biological condition of the patient.

Preferred embodiments of the invention include the use of calibrated profile data bases for determining the biological condition at one site in a subject from a sample taken from a second remote site. The biological condition may include disease, therapeutic interventions, aging, health conditioning and exercise, exposure to toxins, status of infection and health status. For example,

calibrated precision profiles may be used to measure a biological condition(s) in one site (for example, the liver) by sampling cells from the same subject, but at a different site not generally considered a target for the biological condition, for example, peripheral blood cells in the case of liver disease.

Preferred embodiments of the invention include the use of calibrated profile data bases for determining the biological condition of the subject that includes placing a cell or fluid sample on indicator cells to assess the biological condition, the biological condition including disease, therapeutic interventions, aging, health conditioning and exercise, exposure to toxins, status of infection and health status.

Preferred embodiments of the invention include the use of calibrated profile data bases and profiles to assess, compare and contrast the bioactivities of therapeutic agents and therapeutic agent candidates including comparison of two agents having unknown properties ; comparison of agents that are complex mixtures against those that are simple mixtures and comparisons of a single agent against a class of agents.

Preferred embodiments of the invention include the use of calibrated profile databases derived from in vitro dosing of an agent in indicator cells, or fluids or cells ex vivo to predict in vivo activities, activities including efficacy and toxicity and further permitting data on short term in vivo dosing of agent to predict long-term activities as described herein.

A preferred embodiment of the invention is at least one databases and its uses, the databases containing at least one of calibrated profile data sets and baseline profile data sets for discrete populations identified according to factors including diseases, geography, ethnicity, age and state of health.

A preferred embodiment of the invention is a database corresponding to an individual over time, the uses including managing a personalized health care program.

Additional embodiments include methods of running a clinical trial using calibrated profile data and databases containing calibrated profile data from in vitro and in vivo studies of the effect of the agent on populations of cells and

methods of building a clinical research network that uses calibrated profile data and traditional medical data.

Brief Description of the Drawings

The foregoing features of the invention will be more readily understood by reference to the following detailed description, taken with reference to the accompanying drawings, in which:

Figure 1 is a diagram showing the flow of information from data acquired in molecular pharmacology and toxicology, clinical testing, and use of the data for the application to individualized medicine.

Figure 2 is a diagram showing the drug discovery pathway of new compounds from early leads to likely drug candidates. Although calibrated profile data sets are indicated at the pre-clinical step, gene expression data can be acquired and is useful at any of the stages shown. IND refers to investigative new drug and refers to an early stage in regulatory review.

Figure 3 is a diagram presenting a comparison of in vivo and in vitro protocols for forming calibrated profile data sets for rapidly assessing product candidate toxicity and efficacy in accordance with several embodiments of the present invention.

Figure 4 is a diagram showing the application of gene expression profiling as a guide to pre-clinical and clinical studies in accordance with an embodiment of the present invention.

Figure 5 is a diagram showing a method in accordance with an embodiment of the present invention for obtaining profile data in the absence of a stimulus and in the presence of a stimulus.

Figure 6 is a diagram showing the creation of a library of profile data associated with a plurality of subjects in accordance with an embodiment of the present invention.

Figure 7 is a diagram illustrating the structure of a profile data record in accordance with an embodiment of the present invention.

Figure 8 is a diagram illustrating a data entry screen for a data record of the type shown in Figure 7 and typical contexts in which data records may be

compiled in accordance with embodiments of the present invention.

Figure 9 shows an embodiment of the present invention in which profile data, in either the raw or calibrated form, is evaluated using data from a database that is remotely accessed over a network.

Figure 10 shows a schematic of a phase two clinical trial that utilizes gene expression profiling (a). The right hand panel (b) indicates that the same information may be used in Phase IV or post marketing studies to compare the efficacy of already approved and marketed drugs or to guide the marketing of such therapies; to guide the choice of therapy for an individual subject or population from within a class of appropriate compounds.

Figure 11 is a bar graph that shows a graphical representation in the form of a histogram representing calibrated profile data sets based on quantitative expression of RNA in cells of a whole blood sample using a panel of 12 constituents where each constituent corresponds to a unique gene locus. (a) The blood sample is stimulated ex vivo with heat killed staphylococci are further exposed H7-TPCK, H9-UT-77, or H16-Dex as indicated. The baseline profile data set is a blood sample stimulated ex vivo (in vitro) with heat killed staphylococci (b) The blood sample is stimulated ex vivo with lipopolysaccharide (LPS) and is then further exposed to compounds H7-TPCK, H9-UT-77, or H16-Dex as indicated.

Figure 12 is a bar graph with a logarithmic axis that shows a graphical representation of calibrated profile data sets for whole blood stimulated ex vivo with lipopolysaccharide (LPS), using a panel of 9 constituents, each constituent corresponding to a gene locus encoding the gene products indicated, the blood being further exposed to anti-inflammatory agents: methotrexate, meclofenamate and methylprednisolone. The baseline profile data set is derived from LPS stimulated (but otherwise untreated) cells.

Figure 13 are bar graphs with a logarithmic axis that shows a graphical representation of calibrated profile data sets for two different samples of whole blood (a) 991116 and (b) 991028 reflecting the biological condition of the cells using a panel of 24 members, each member corresponding to a gene locus, the

baseline profile data set being derived from untreated cells. The calibrated data sets for cells exposed for six hours to three inflammation inducing agents (lipopolysaccharide, heat killed staphylococci, and phytohemaglutinin) are compared for each sample. (c) shows a direct comparison of LPS stimulated 991116 with respect to 991028 as the baseline profile data set (d) shows a direct comparison between unstimulated 991116 and 991028.

Figure 14 is a bar graph with a logarithmic axis that shows a graphical representation of calibrated profile data sets using a panel of 22 constituents, each constituent corresponding to a gene locus, the baseline profile data set being derived from untreated cells. Whole blood is exposed for six hours ex vivo to three inflammation inducing agents (lipopolysaccharide, heat killed staphylococci, and phytohemaglutinin) which are then treated with a single anti-inflammatory agent (methyl prednisolone) to reveal similarities and differences in the effect of a single agent on cell populations differing in their biological condition.

Figure 15 is a bar graph with a logarithmic axis that shows a graphical representation of calibrated profile data sets for whole blood where one calibrated data set refers to a subject (subject 2) who has been treated in vivo with a corticosteroid (dexamethasone), a second data set refers to the treatment of a blood sample from the same subject prior to in vivo treatment where that sample has been treated ex vivo (in vitro) and the third data set refers to a second subject treated in vivo with dexamethasone (subject 1). The data sets demonstrate the reproducibility and predictability of an *ex vivo* (in vitro) treatment of blood compared to *in vivo* treatment with the same agent. The figure also shows minor variation between samples from different subjects reflecting interpersonal variability. A panel of 14 constituents is provided. The baseline profile data set is derived from untreated whole blood from the cognate subject.

Figure 16 is a bar graph with a logarithmic y axis that shows a graphical representation of calibrated profile data sets for whole blood where one calibrated data set refers to (a) 2 subjects who have been treated in vivo with an inactive placebo for 3 days and (b) active prednisolone for 3 days at 100 mg/day.

The data set shows some variation between samples from different subjects treated with the same drug. The data sets demonstrate similarity of responses across the same gene loci, as well as, quantitative variation at other loci suggesting quantifiable interpersonal variation. A panel of eight members is provided. The baseline profile data set is derived from untreated whole blood.

Figure 17 is a bar graph with logarithmic y axis that shows a graphical representation of calibrated precision profile data sets for two samples taken from a single subject within a 19 day period using a panel (e.g., inflammation panel) of 24 members where each member corresponds to a unique gene locus. The baseline profile data set relates to peripheral blood taken from the subject prior to treatment.

Figure 18 (a-e) are bar graphs with a logarithmic axis that show a graphical representation of calibrated profile data sets for each of 5 subjects from which a blood sample has been taken. Each of the blood samples was exposed to the inflammatory agent phytohemagglutin (PHA) or to a therapeutic agent (anti-inflammatory agent) at different concentrations: 0.1μM, 0.3uM, 1μM, 3μM and 5μM, for a 4 hour period ex vivo (in vitro) so as to determine the optimum dose for treating the subject. A panel of 6 constituents were used corresponding to 6 gene loci. The baseline profile data set was untreated sample obtained from the cognate donor.

Figure 19 is a bar graph with a logarithmic axis that shows a graphical representation of calibrated profile data sets for three different subjects having different biological conditions using a panel with 24 constituents. The profile data sets show variability according to these conditions providing the basis for a diagnostic signature panel. (a) shows a calibrated profile data set for a smoker against a baseline for a non-smoker. (b) shows a calibrated profile data set for a subject with chronic obstructive pulmonary disease against a baseline for a subject lacking this disease. The baseline profile data set is derived from a subject that is "normal" with respect to these conditions.

Figure 20 illustrates that an individual responses can be distinguished from a similarly treated population. A comparison of the response of a single

animal compared to its experimental cohort (n=5 animals) with respect to a single locus (GST-P) is provided. The baseline data set is the cohort average. The figures shows that this animal varied significantly from the daily, population average in the first two days of the study, but became more similar to the cohort average with time after treatment with acetaminophen.

Figure 21 is a bar graph with a logarithmic axis that shows a graphical representation of calibrated profile data sets for samples of blood treated ex vivo with LPS or LPS and one of three anti-inflammatory herbals (Echinacea, Arnica or Siberian Ginseng) at a concentration of 200 ug/ml. A panel of 24 constituents is used. The baseline profile data set is derived from LPS stimulated cells absent a herbal treatment. The figure illustrates the effectiveness of the use of the calibrated precision profile to investigate the overall effects of complex compounds such as nutraceuticals whose biological effect is a summation of more than one activity. In this case, each of the herbals is consumed as an immunostimulant, however the calibrated precision profiles reveal a unique pattern shows a mixture of both immunostimulatory and anti-inflammatory effects.

Figure 22 is a bar graph with a logarithmic axis that shows a graphical representation of calibrated profile data sets for samples of blood treated ex vivo with LPS or LPS and methylprednisolone or LPS and Arnica. The baseline profile data set is LPS treated blood sample.

Figure 23 is a bar graph with a logarithmic axis that shows a graphical representation of calibrated profile data sets for samples of THP-1 cells treated with LPS or LPS and Arnica at three different concentrations using a panel of 22 constituents. The baseline profile data set is untreated THP-1 cells. The figure illustrates a concentration response with respect to the gene expression across the calibrated profile.

Figure 24 is a bar graph with a logarithmic axis that shows a graphical representation of calibrated profile data sets for samples of THP-1 cells treated ex vivo with four different commercial brands of Echinacea using a panel of 8 constituents. The baseline profile data set is untreated THP-1 cells.

Figure 25 illustrates the use of the calibrated profile to compare relative efficacy across brands, or different formulations. Calibrated profile data sets for herbal preparations from different manufacturing sources with respect to an indicator monocytic cell line (THP-1) are shown graphically, the baseline profile data set being THP-1 cells absent the herbal. (a) three commercial herbal Echinacea preparations at 250 (ug/ml); (b) three herbal preparations at different concentrations (250ug/ml, 50ug/ml and 3-10ug/ml) (c) four commercial Echinacea brands at 250 ug/ml).

Detailed Description of Specific Embodiments

As used in this description and the accompanying claims, the following terms shall have the meanings indicated, unless the context otherwise requires:

A "collection of cells" is a set of cells, wherein the set has at least one constituent.

A "population of cells" includes one or more cells. A population of cells may refer to cells *in vivo* or to *in vitro* cultures. *In vitro* cultures may include organ cultures or cell cultures where cell cultures may be primary or continuous cell cultures of eukaryotic or prokaryotic cells. Cell lines can be primary cultures or cell samples, e.g. from a tumor, from blood or a blood fraction, or biopsy explants from an organ, or can be established cell lines or microbial strains.

A "region of the subject" from which proteins are obtained may (but is not required to be) the same part of the subject from which has been obtained a collection of cells or a population of cells. The cells and the proteins may both be obtained from blood of the subject, for example. Alternatively, for example, the cells may be obtained from blood and the proteins may be obtained from a scraping of tissue or vice versa. Similarly, the proteins may be obtained from urine of the subject, for example, whereas the cells may obtained elsewhere, as, for example, from blood.

A "panel" of genes is a set of genes including at least two constituents.

A "normative" condition of a subject to whom a composition is to be administered means the condition of a subject before administration, even if the subject happens to be suffering from a disease.

An "expression" of a gene includes the gene product whether messenger RNA or protein resulting from translation of the messenger RNA.

A "large number" of data sets based on a common panel of genes is a number of data sets sufficiently large to permit a statistically significant conclusion to be drawn with respect to an instance of a data set based on the same panel.

A "biological condition" of a subject is the condition of the subject in a pertinent realm that is under observation, and such realm may include any aspect of the subject capable of being monitored for change in condition, such as health, disease including cancer; trauma; aging; infection; tissue degeneration; developmental steps; physical fitness; obesity, or mood. As can be seen, the conditions may be chronic or acute or simply transient. Moreover, a targeted biological condition may be manifest throughout the organism or population of cells or may be restricted to a specific organ (such as skin, heart, eye or blood). The term "biological condition" includes a "physiological condition"

The "blind administration" of a selected one of a composition or placebo to a subject in a clinical trial involves administering the composition or placebo to the subject in accordance with a protocol pursuant to which the subject lacks knowledge whether the substance administered is the composition or a placebo.

An "organism" is any living cell including microorganisms, animals and plants. An animal is commonly in this context a mammal, but may be a vertebrate non-mammal, as e.g., a zebra fish, or an invertebrate, as, e.g. *Caenorhabditis elegans*.

An "agent" is a composition or a stimulus. A "stimulus" may include, for example ultraviolet A or B, or light therapy for seasonal affective disorder, or treatment of psoriasis with psoralen or treatment of melanoma with embedded radioactive seeds, other radiation exposure, etc. A "composition" includes a chemical compound, a nutraceutical, a combination of compounds, or a complex mixture.

A "clinical indicator" is any physiological datum used alone or in conjunction with other data in evaluating the physiological condition of a

collection of cells or of an organism. This term includes pre-clinical indicators.

A "signature panel" is any panel representing a subclass of constituents where the subclass of constituents is selected according to the relatively high level of information concerning a biological condition imparted by each member of the data set.

"Distinct RNA or protein constituent" in a panel of constituents is a panel that includes at least one of RNA and protein and each constituent of the panel is distinct.

A preferred embodiment of the invention is the formation of calibrated data sets that describe a biological condition or an effect of an agent on a biological condition. A calibrated data set represents a set of values that correspond to variations in gene expression where the variations are informative. This approach does not require comprehensive analysis of all gene expression in target cells associated with a particular condition. Nor is any one single gene locus necessarily of particular significance. Rather a pattern of variation (a profile) is sought that correlates, in a reproducible manner, with a particular condition. There may be no *a priori* knowledge of a correlation but rather a correlation may be established by evaluating a panel of constituents of reasonable size (for example up to 100 constituents) and iteratively testing the gene expression profiles for different subjects or for the same subject from which the most informative loci for a particular condition may be selected. An informative subgroup of constituents in a panel may be selected that consistently vary for a particular condition and this subgroup may then become the signature panel, the signature panel giving rise to a signature profile.

In further embodiments of the invention, any calibrated data set for an individual that has more members than reflective of a single signature panel may be mined for calibrated profiles that correspond to additional signature panels thereby potentially providing new insights into mechanisms of action of a biological condition on sets of genes. Measurement of changes in transcribed RNA in a cell as a result of an environmental change or aging is an exquisitely sensitive measure of the response of a cell. Techniques available today to

quantify transcribed RNA in a cell add to the sensitivity of the approach. The preferred embodiments of the invention, which are directed to patterns of change in amounts of transcribed RNA, provide a means to focus and interpret this rich information.

In contrast to the above approach, much attention in the prior art has been directed to the sequencing of the human genome and the identification of all the genes encoded therein. Accompanying the growing amount of sequence data, microarrays provide a means to survey thousands of gene sequences for mutations. Microarrays are being used to provide DNA profiles that identify mutations in an individual and those mutations will be associated with predictions concerning development of disease in those individuals. Transcriptomics and proteomics is now the focus of increasing attention. These studies are directed to analyzing the entire body of RNA and protein produced by living cells. Microarrays provide a method for analyzing many thousands of different human RNAs as to whether they are expressed and by which cells. For example, a project undertaken by the National Cancer Institute and others to examine mRNAs produced by various types of cancer cells, have revealed 50,000 genes that are active in one or more cancers. The goal of these studies is to identify novel cancer drugs that are directed to knocking out or enhancing the production of certain proteins. (Kathryn Brown, The Human Genome Business Today, Scientific American, July 2000, p.50; Julia Karow, The "Other" Genomes, Scientific American, July 2000, p.53; Ken Howard, "The Bioinformatics Gold Rush, Scientific American, July 2000, p.58; Carol Ezzell, Beyond the Human Genome, Scientific American, July 2000, p.64; all incorporated by reference). Major efforts in correlating genetic variation of individuals and the functional interrelationships of genes in health and disease are being conducted in a variety of consortia including the single nucleotide polymorphism consortium and the Human Epigenome Consortium (Beck et al. Nature BioTechnology 17 (1999) p 1144). The Epigenome Consortium plans to analyze sets of genome fragments from both healthy and diseased individuals in the 500 different human tissues (Bioworld International: December 22, 1999). This approach seeks to correlate

absolute expression of genes associated with a particular condition with the presence of that condition. Examples of prior art that seek to measure gene expression in absolute amounts including by subtractive methods or by determining amounts with respect to housekeeping genes or by targeting a single gene expression system are U.S. 5,643,765; U.S. 5,811,231; U.S. 5,846,720; U.S. 5,866,330; U.S. 5,968,784; U.S. 5,994,076; WO 97/41261; WO 98/24935; WO 99/11822; WO 99/44063; WO 99/46403; WO 99/57130; WO00/22172 and WO00/11208.

We have taken a different and novel approach to the above by identifying reproducible patterns of variation of gene expression that are informative by virtue of the degree of variation between a sample and a baseline for example a subject with the condition and the subject that lacks the condition. The variations may be correlated with other non- genetic indications such as clinical indicators (for humans) of a traditional nature but are not required per se to be causative. Accordingly, the amount of gene expression product (for example RNA transcript) produced by a gene locus in a cell under certain circumstances is measured and then stored as a value in a first profile data set. This value is calibrated with respect to a second value (a baseline profile data set) to provide a member of a calibrated profile data set. The values recorded for the profile data set, relying on a particular baseline data set to produce a calibrated data set become part of the descriptive record any or all of which can be stored in a database which may be accessed through a global network such that any new data in the form of a profile data set or a calibrated profile data set measured at any global location can be directly compared to an archive of descriptive records including calibrated profile data sets and baseline data sets so as to extend the stored library of profiles and provide predictive or diagnostic data about a particular biological condition or agent.

We have exemplified the use of selected panels of constituents corresponding to gene loci from which quantitative gene expression is measure by for example quantitatively measuring the transcribed RNA in a sample of a subject, for applications that include: (a) measurement of therapeutic efficacy of

natural or synthetic compositions or stimuli that may be formulated individually or in combinations or mixtures for a range of targeted physiological conditions; (b) predictions of toxicological effects and dose effectiveness of a composition or mixture of compositions for an individual or in a population; (c) determining how two different agents administered in a single treatment might interact so as to detect any of synergistic , additive, negative, neutral of toxic activity (d) performing pre-clinical and clinical trials by providing new criteria for pre-selecting subjects according to informative profile data sets for revealing disease status and conducting preliminary dosage studies for these patients prior to conducting phase 1 or 2 trials. Gene expression profiling may be used to reduce the cost of phase 3 clinical trials and may be used beyond phase 3 trials; (e) labeling for approved drugs; (f) selection of suitable medication in a class of medications for a particular patient that is directed to their unique physiology; (g) diagnosing or determining a prognosis of a medical condition or an infection which may precede onset of symptoms or alternatively diagnosing adverse side effects associated with administration of a therapeutic agent; (h) managing the health care of a patient; and (i) quality control for different batches of an agent or a mixture of agents.

The subject

The methods herein can be applied to a subject that includes any living organism where a living organism includes a prokaryote such a bacterium or a eukaryote including single celled eukaryotic organisms at one end of the spectrum and humans at the other and everything in between including plants. The figures relate to calibrated profile data sets obtained from humans and mammals. Nonetheless, the methods disclosed here may be applied to cells of other organism without the need for undue experimentation by one of ordinary skill in the art because all cells transcribe RNA and it is know in the art how to extract RNA from all types of cells.

A tissue sample might include a single cell or multiple cells or fragments of cells. Body fluid includes blood, urine, spinal fluid, lymph, mucosal secretions, hemolymph or any other body fluid known in the art for a subject.

For an animal subject, a tissue or fluid sample may be obtained by means of a biopsy needle aspirate, a lavage sample, scrapings and surgical incisions or other means known in the art.

Panels

Steps in selecting constituents in a panel include searching publicly available medical literature for RNA or proteins or sets of RNAs or proteins that directly or indirectly vary with a particular biological condition. A panel containing up to 100 constituents may be selected. According to the condition being examined, just a small subset of the panel constituents may be informative. In determining membership of the panel of genes, it is not necessary for the panel to be an exhaustive selection. Rather it is desired to obtain from the panel an expression profile that discriminates consistently with respect to the targeted physiological or biological condition. Moreover, a panel is not necessarily selected according to an expected profile of gene expression in cells that directly respond to a biological effect. For example, gene expression associated with liver metabolism may be analyzed in a blood sample. Figures 20 and 22 provide calibrated profiles of whole blood treated with herbal agents using markers for liver metabolism.

The number of constituents in a panel can vary. According to the examples provided below, panels of up to 24 genes are selected for evaluating expression levels. Although a panel may be as large as 100 constituents, it is desirable for a particular panel to have no more than 24 constituents, more particularly, less than 12 constituents. For example, subsets of no more than 8 genes have been used that may be derived from a larger panel but which are sufficiently informative to effectuate discrimination. The number of constituents in a panel for which expression is monitored may vary widely depending on the context. For example, Figure 1 describes data acquisition from *in vitro* cell culture and from animal toxicology studies, which includes expression of about 25 to 100 or more genes. In contrast, selection of markers or surrogate markers include for example three to 100 genes, preferably five to 50 or five to 25 genes to be analyzed from samples obtained in clinical studies. In this manner markers or

surrogate markers having predictive value for a medical condition, such as a genetic predisposition, a response to therapeutic agent, an inflammatory condition, or an infection, etc. can be identified and cumulatively larger populations can be obtained to refine the correlations. A health profile can then be generated for an individual subject using a low volume blood sample. The blood sample can be analyzed for expression profile data of about 100 - 500 genes, comprising markers or surrogate markers of a number of medical conditions (Fig. 1: right panel). Panels of varying sizes may be utilized as necessary and subsequent refinements in methodology may lead to selection of subsets having panels as large as 15 genes or 12 genes or as small as 6, 5, 4, 3 or 2 genes.

It is envisaged that any single biological condition may be described by a signature panel having a small number of highly informative constituents providing a signature calibrated profile (also referred to as a fingerprint). The presence of highly informative loci is demonstrated in several of the accompanying figures. For example, Figure 11(a) Il-2, Il-4 and Il-5 appeared to be highly informative. Highly informative constituents in Figure 21 include the interleukins. The signature panel may provide a signature profile or fingerprint which is sufficiently robust to serve as a standard in describing a particular biological condition or an effect of a particular agent on a biological condition.

For purposes of illustrating a signature panel, constituents of a panel for measuring inflammation have been provided that are informative with respect to a particular biological condition. For example, we have used a panel for inflammation that has 6 constituents- Il-1a, Il-6, Il-8, Il-18, GMCSF and IFN-g in Figure 18(a)–(e) to determine the response of 5 subjects to varying concentrations of drugs. This group of constituents is a subset of a larger panel of inflammation related gene loci such as shown in Figure 19a and Figure 19b where the Inflammatory Panel includes Il-a, Il-b, Il-2, Il-3, Il-4, Il-6, Il-7, Il-8, Il-10, Il-12p40, Il-15, Il-15, Il-18, GM-CSF, Ifn-gamma, TGF-b, cox-2, ICE, MMP-9, ICAM, TNF-a and TNF-b. The subset of constituents were selected on the basis of the information sought concerning the biological condition.

Embodiments of the invention provide examples of at least 4 different panels which may be used separately or together. These panels are an inflammatory panel (TNF-a, Il-1b, ICAM, Il-8, Il-10, Il-12p40, ICE, cox-2, cox-1 and mmp-3) a cell growth and differentiation panel (c-fos, c-jun and STAT3), a toxicity panel (SOD-1, TACE, GR, HSP70, GST, c-fos, c-jun, INOS) and a liver metabolism panel (INOS, cyp-a and u-pa). Other panels include skin response or prostate cancer or endothelial/cardiovascular response panels or cell growth or differentiation or liver metabolism panels. Although provided as examples, the above panels are not intended to be limiting.

Gene Expression

For measuring the amount of a particular RNA in a sample, we have used methods known to one of ordinary skill in the art to extract and quantify transcribed RNA from a sample with respect to a constituent of a panel. RNA is extracted from a sample such as a tissue, body fluid, or culture medium in which a population of a subject might be growing. For example, cells may be lysed and RNA eluted in a suitable solution in which to conduct a DNAse reaction. First strand synthesis may then performed using a reverse transcriptase. Gene amplification, more specifically quantitative PCR assays, can then conducted and the gene of interest size calibrated against a marker such as 18S rRNA (Hirayama et al., Blood 92, 1998: 46-52). Samples are measured in multiple duplicates for example, 4 replicates. Relative quantitation of the mRNA is determined by the difference in threshhold cycles between the size marker and the gene of interest. In an embodiment of the invention, quantitative PCR is performed using amplification, reporting agents and instruments such as those supplied commercially by PE Biosystems (Foster City, CA). Given a defined efficiency of amplification of target transcripts, the point (e.g., cycle number) that signal from amplified target template is detectable may be directly related to the amount of specific message transcript in the measured sample. Similarly, other quantifiable signals such as fluorescence, enzyme activity, disintegrations per minute, absorbance, etc., when correlated to a known concentration of target templates (e.g., a reference standard curve) or normalized to a standard with limited

variability can be used to quantify the number of target templates in an unknown sample.

Although not limited to amplification methods, quantitative gene expression techniques may utilize amplification of the target transcript. Alternatively or in combination with amplification of the target transcript, amplification of the reporter signal may also be used. Amplification of the target template may be accomplished by isothermic gene amplification strategies, or by gene amplification by thermal cycling such as PCR. It is desirable to obtain a definable and reproducible correlation between the amplified target or reporter and the concentration of starting templates.

It is envisaged that techniques in the art using microfluidics for example and highly sensitive markers will enable quantitation of RNA to occur directly from a single cell or lysed cell. This may rely on amplification of a marker but may not require amplification of the transcripts themselves. The amount of transcript measured for any particular locus is a data point or member of the first profile data set for a particular panel.

According to embodiments of the invention, a first profile data set is derived from the sample, the first profile data set including a plurality of members, each member being a quantitative measure of the amount of a RNA transcribed from a gene locus, the gene locus being a constituent in a panel of constituents. A first profile data set may be obtained from a quantitative measure of the amount of a distinct RNA or protein corresponding to a gene locus. The figures provided here are directed to RNA. However, the method could be applied using proteins where sensitive quantitative techniques are available for measuring the amount of a distinct protein in a cell.

Baseline profile data sets

The analyses of samples from single individuals and from large groups of individuals provide a library of profile data sets relating to a particular panel or series of panels. These profile data sets may be stored as records in a library for use as baseline profile data sets. As the term "baseline" suggests, the stored baseline profile data sets serve as comparators for providing a calibrated profile

data set that is informative about a biological condition or agent. It is anticipated that many baseline profile data sets will be stored in libraries and classified in a number of cross-referential ways. One form of classification might rely on the characteristics of the panels from which the data sets are derived. Another form of classification might be the use of a particular biological condition. The concept of biological condition encompasses any state in which a cell or population of cells might be at any one time. This state might reflect geography of samples, sex of subjects or any other discriminator. Some of the discriminators may overlap. The libraries might also be accessed for records associated with a single subject or particular clinical trial. The classification of baseline profile data sets may further be annotated with medical information about a particular subject, a medical condition, a particular agent etc.

The choice of a baseline profile data set for creating a calibrated profile data set is related to the biological condition to be evaluated, monitored, or predicted, as well as, the intended use of the calibrated panel, e.g., as to monitor drug development, quality control or other uses. It might be desirable to access baseline profile data sets from the same subject for whom a first profile data set is obtained or from different subject at varying times, exposures to stimuli, drugs or complex compounds; or may be derived from like or dissimilar populations.

The profile data set may arise from the same subject for which the first data set is obtained, where the sample is taken at a separate or similar time, a different or similar site or in a different or similar physiological condition. For example, Figure 5 provides a protocol in which the sample is taken before stimulation or after stimulation. The profile data set obtained from the unstimulated sample may serve as a baseline profile data set for the sample taken after stimulation. The baseline data set may also be derived from a library containing profile data sets of a population of subjects having some defining characteristic or biological condition. The baseline profile data set may also correspond to some ex vivo or in vitro properties associated with an in vitro cell culture. The resultant calibrated profile data sets may then be stored as a record in a database or library (Figure 6) along with or separate from the baseline profile

data base and optionally the first profile data set although the first profile data set would normally become incorporated into a baseline profile data set under suitable classification criteria.

Selected baseline profile data sets may be also be used as a standard by which to judge manufacturing lots in terms of efficacy, toxicity, etc. Where the effect of a therapeutic agent is being measured, the baseline data set might correspond to gene expression profiles taken before administration of the agent. Where quality control for a newly manufactured product is being determined, the baseline data set might correspond with a gold standard for that product. However, any suitable normalization techniques may be employed. For example, an average baseline profile data set is obtained from authentic material of a naturally grown herbal nutraceutical and compared over time and over different lots in order to demonstrate consistency, or lack of consistency, in lots of compounds prepared for release.

Calibrated data

A calibrated profile data set may be described as a function of a member of a first profile data set and a corresponding member of a baseline profile data set for a given gene locus in a panel. For example, calibrated profile data sets may be derived by calculating a ratio of the amount of RNA transcribed for a panel constituent in a cell sample in an environmental including intervention such as a therapeutic treatment or at a particular time (first profile data set) with respect to the amount of RNA transcribed for the same panel constituent in a cell that differs in some manner from the sample (baseline profile data set) (Figures 5 and 6). We have found that calibrated profile data sets to be reproducible in samples that are repeatedly tested (Figure 17). We have also found that calibrated profile data sets obtained when samples from a subject are exposed ex vivo to a compound are comparable to calibrated profile data from a sample that has been exposed to a sample in vivo (Figure 14 and Figure 16(a),(b)). We have also found that an indicator cell line treated with an agent can provide comparable calibrated profile data sets to those obtained from in vivo or ex vivo populations of cells (Figure 15). Moreover, we have found that administering a sample from

a subject onto indicator cells can provide informative calibrated profile data sets with respect to the biological condition of the subject including the health, disease states, therapeutic interventions, aging or exposure to environmental stimuli or toxins of the subject (Figure 25).

A preferred use of a calibrated profile data set is to evaluate a biological condition of a subject. This may be for purposes of diagnosis or prognosis of a clinical disorder. It is desirable to obtain a calibrated data set that describes a state of health or alternatively a state of age or body mass or any condition or state that an individual subject might find themselves to be in. For example, the biological condition might relate to physical activity, conditioning or exercise, mental state, environmental factor such as medication, diet, or geography or exposure to radiation or environmental contamination or infectious agent, biological or environmental toxin. If health or conversely a clinical disorder is being evaluated, calibrated profiles data sets may be used for monitoring change in health status by periodic or regular comparison of profiles; the disorder may be a complex disease process possibly involving multiple gene including inflammation, autoimmune disease, degenerative disease, allergy, vascular disease, ischemia, developmental disease, hormonal conditions and infectious diseases. The clinical disorder may further include arthritis, asthma, multiple sclerosis and perimenopausal changes. The biological condition may affect a system of a subject including a respiratory, vascular, nervous, metabolic, urinary, reproductive, structural and immunological system or other metabolic state. The above examples of a biological condition are given by way of illustration and are not intended to be limiting.

Similarly, calibrated profile data sets can be used to measure, monitor or predict the host response to an infectious agent for purposes of identifying the infectious agent, assessing the duration of infection, the extent of exposure or making therapeutic decisions.

The evaluation of activity of an agent may require a series of calibrated profiles. It is here shown that calibrated profile data sets can be used to describe the biological activity of an agent that might be a single compound or a complex

compound such as a nutraceutical or herbal. The agent can be assayed using indicator cells, ex vivo cell populations or by in vivo administration. These assays may rely on a series of signature panels or enlarged panels for different biological conditions. The resultant calibrated profiles may then be used to infer likely in vivo activity from the in vitro study. Insights into toxicity and mechanisms of action can also be inferred from calibration profile data sets. For example, the herbal Echinacea is believed to have both immunostimulatory and anti-inflammatory properties although neither has been measured systemmatically. We have provided a systematic approach to investigate the biological activities of these and other herbs. We investigated the alleged immunostimulatory properties of the herbs by comparing the effect of treating the indicator cell line THP-1 or peripheral blood cells with the agent to untreated cells. Untreated cells include LPS stimulated untreated cells. Untreated cells were used as a baseline profile data set to measure the difference in gene expression between a baseline profile data set and the experimental treatment with the compound. Baseline profile data sets included a single sample or an average value from a series of experiments. The resultant calibrated profile data sets could then be compared with a library of calibrated profile data sets for a particular herb or/and libraries associated with different agents or conditions.

From the information obtained about a previously undescribed agent, a signature panel may be derived optionally together with a signature profile to serve as a gold standard for testing other batches of the same agent.

Calculation of calibrated profile data sets and computational aids

The function relating the baseline and profile data sets is in a preferred embodiment, a ratio expressed as a logarithm. The calibrated profile data set may be expressed in a spreadsheet or represented graphically for example, in a bar chart or tabular form but may also be expressed in a three dimensional representation. Preferably the constituent is itemized on the x-axis and the logarithmic scale is on the y-axis. Members of a calibrated data set may be expressed as a positive value representing a relative enhancement of gene expression or as a negative value representing a relative reduction in gene

expression with respect to the baseline.

Each member of the calibrated profile data set should be reproducible within a range with respect to similar samples taken from the subject under similar conditions. For example, the calibrated profile data sets may be reproducible within one order of magnitude with respect to similar samples taken from the subject under similar conditions. More particularly, the members may be reproducible within 50% more particularly reproducible within 20%. Each member of the calibrated profile data set has a biological significance if it has a value differing by more than an amount D, where D=F(1.1)-F(.9) and F is a second function.

It is the pattern of increasing, decreasing and no change in gene expression from the plurality of gene loci examined in the panel that is used to prepare a calibrated profile set that is informative with regards to a biological condition, biological efficacy of an agent treatment conditions or for comparison to populations and which may be used to identify likely candidates for a drug trial, used in combination with other clinical indicators to be diagnostic or prognostic with respect to a biological condition or may be used to guide the development of a pharmaceutical or nutraceutical through manufacture, testing and marketing.

The numerical data obtained from quantitative gene expression and numerical data from calibrated gene expression relative to a baseline profile data set may be stored in databases or digital storage mediums and may retrieved for purposes including managing patient health care or for conducting clinical trials or for characterizing a drug. The data may be transferred in networks via the World Wide Web , email, or internet access site for example or by hard copy so as to be collected and pooled from distant geographic sites (Figure 8).

In a preferred embodiment, a descriptive record is stored in a single or multiple databases where the stored data includes the raw gene expression data (first profile data set) prior to transformation by use of a baseline profile data set, as well as a record of the baseline profile data set used to generate the calibrated profile data set including for example, annotations regarding whether the

baseline profile data set is derived from a particular signature panel and any other annotation that facilitates interpretation and use of the data.

Because the data is in a universal format, data handling may readily be done with a computer. The data is organized so as to provide an output optionally corresponding to a graphical representation of a calibrated data set. For example, a distinct sample derived from a subject being at least one of RNA or protein may be denoted as P_1. The first profile data set consists of M_j where Mj is a quantitative measure of a distinct RNA or protein constituent. The record Ri is a ratio of M and P and may be annotated with additional data on the subject relating to for example, age, diet, ethnicity, gender, geographic location, medical disorder, mental disorder, medication, physical activity, body mass and environmental exposure. Moreover, data handling may further include accessing data from a second condition database which may contain additional medical data not presently held with the calibrated profile data sets. In this context, data access may be via a computer network.

The above described data storage on a computer may provide the information in a form that can be accessed by a user. Accordingly, the user may load the information onto a second access site including downloading the information. However, access may be restricted to users having a password or other security device so as to protect the medical records contained within. A feature of this embodiment of the invention is the ability of a user to add new or annotated records to the data set so the records become part of the biological information.

The graphical representation of calibrated profile data sets pertaining to a product such as a drug provides an opportunity for standardizing a product by means of the calibrated profile, more particularly a signature profile. The profile may be used as a feature with which to promote the drug.

The various embodiments of the invention may be also implemented as a computer program product for use with a computer system. The product may include program code for deriving a first profile data set and for producing calibrated profiles. Such implementation may include a series of computer

instructions fixed either on a tangible medium, such as a computer readable media (for example, a diskette, CD-ROM, ROM, or fixed disk), or transmittable to a computer system via a modem or other interface device, such as a communications adapter connected to a network over a medium. The medium may be either a tangible medium (for example, optical or analog communications lines) or a medium implemented with wireless techniques (for example, microwave, infrared or other transmission techniques). The series of computer instructions preferably embodies all or part of the functionality previously described herein with respect to the system. Those skilled in the art should appreciate that such computer instructions can be written in a number of programming languages for use with many computer architectures or operating systems. Furthermore, such instructions may be stored in any memory device, such as semiconductor, magnetic, optical or other memory devices, and may be transmitted using any communications technology, such as optical, infrared, microwave, or other transmission technologies. It is expected that such a computer program product may be distributed as a removable medium with accompanying printed or electronic documentation (for example, shrink wrapped software), preloaded with a computer system (for example, on system ROM or fixed disk), or distributed from a server or electronic bulletin board over the network (for example, the Internet or World Wide Web). In addition, a computer system is further provided including derivative modules for deriving a first data set and a calibration profile data set.

Clinical trials

The use of calibrated profile data sets for performing clinical trials is illustrated in Figure 10 using the above-described methods and procedures for running a clinical trial or managing patient care. Moreover, standardization between laboratories may be achieved by using a particular indicator cell line such as THP-1 which is stimulated by a known stimulator such as lipopolysaccharide so that resultant profile acts as a measure that the laboratory is performing the protocol correctly.

Examples of how embodiments of the invention may be used for

augmenting clinical trials includes providing new methods for patient selection. Clinical trials in which candidate subjects are included or excluded according to a predetermined optimum calibrated profile for a given biological condition can result in more precise monitoring than would be otherwise possible. It can also result in a greater efficiency in clinical trial design because unsuitable patients that have for example complicating factors or conditions can be screened out. The calibrated profile data will also enhance the "signal to noise" by removing non-responders from double blind placebo studies. The basic structure of a clinical trial design using gene expression profiling could follow any of several formats. These include testing body fluid from a candidate patient in the trial ex vivo against a new therapeutic agent and analyzing the calibrated profiles with respect to an agent-treated and placebo-treated samples using a predetermined panel and evaluating whether the candidate patient would be likely to respond without adverse effects to the composition being tested. In selected indications, profile data obtained from *in vitro* cell cultures or organ cultures may be desired where the cell originates from a target subject or from another subject or from an established cell line, or from a cell samples removed from the target subject where the cell samples may be obtained from any body fluid including a blood, urine, semen, amniotic, or a cerebrospinal fluid sample, or from a scraping from mucosal membranes such as from the buccal cavity, the eye, nose, vagina or by means of a biopsy including epithelial, liver, sternum marrow, testicular, or from tumor tissue removed surgically from a tumor at any location. The above-described sources of samples are applicable to any medical use in which calibrated profile data sets are desired.

In vitro dosage and toxicity studies using calibrated profile data sets obtained from indicator cell lines or samples of the patient tested ex vivo can provide useful information prior to initiation of the clinical trial and can significantly reduce the cost and time of a clinical trial while increasing the likelihood of identifying the presence of beneficial effect. In particular, the dose can be optimized on an individualized basis to maximize the impact on therapeutic outcome. For example, Figure 12 shows how ex vivo blood cells

respond to the stimulatory effect of LPS and the subsequent treatment with an anti-inflammatory drug (methotrexate, meclofenamate or methylprednisolone). The data show how the effect of methotrexate and meclofenamate generates similar calibrated profile data sets where the baseline is LPS treated blood. In contrast, the methylprednisolone has a substantially different effect from the other two compounds. A similar type of analysis can be performed with complex mixtures as illustrated in Figure 21 in which the calibrated profiles obtained when Echinacea, Arnica and Siberian Ginseng applied to LPS stimulated blood ex vivo are compared. In this example, all three agents appear to act differently from each other with respect to a sample from a single subject. Similar analyses can be used to compare compounds with unknown targets or activities or metabolic patterns to compounds, complex or simple, with known or pre-determined profiles.

The above methods and procedures can be utilized in the design and running of clinical trials or as a supplemental tool. Moreover, the above methods and procedures can be used to monitor the patients' health as well as the patient's responsiveness to an agent before during and after the clinical trial. This includes monitoring whether multiple agents interfere with each other, act synergistically or additively or are toxic or neural with respect to each other. This type of information is very important as individuals take an increasing number of medications.

Similarly, the methods and procedures described above may be used to manage patient care for an individual or a population. Such methods and procedures may also be used to develop a regional or global research network that uses calibrated profile data sets and the resulting databases to conduct research or trials.

Both the calibration profile data sets in graphical form and the associated databases together with information extracted from both are commodities that can be sold together or separately for a variety of purposes. For example, graphic representations of calibration profile data sets can provide a description of a product with respect to its activity that may be used to promote the product.

Alternatively, the graphical form of the calibrated profile data sets and access to baseline profile databases provide a means for manufacturers to test discrete batches of product against a gold standard.

The data can be used strategically for design of clinical trials. It can also be useful for physicians practicing at remote sites to offer personalized healthcare to a patient. Accordingly, the physician might set up personalized databases for calibrated profile data sets prior to and after treatment of a particular condition. New data on the subject could be added to the personalized database at each visit to the doctor. The data could be generated at remote sites by the use of kits that permit a physician to obtain a first profile data set on a sample from a patient. For remote users to access the site, it is envisaged that secured access to the global network containing libraries of baseline profile data sets and calibrated profile data sets, classified by particular criteria and representing data from larger populations than a single individual, would be necessary. The access to the global database may be password protected thereby protecting the database from corrupted records and safeguarding personal medical data. The graphical form provided by the calibrated data sets may be used to create catalogs of compounds in a pharmacopiae complete with toxic effects that might arise for particular individuals as well as other types of drug interactions.

Access to the global data base may include the option to load selected data onto a second access site. This process could include downloading the information to whatever site is desired by the user and could include securing hard copies of information. It is desirable to control how and what data is offloaded or dopied to maintain the integrity of the database. It is envisaged that while a global network of clinical data would be an informational resource, it would have utility is conducting research that might include epidemiological studies and studies concerning the mechanism of action of an agent and studies concerning the nature of interpersonal variability as determined by calibrated profile data sets.

Examples of Medical uses

(a) Early detection of infectious diseases: Markers or surrogate

markers from mice may be obtained for measuring gene expression in humans that indicate early or immediate response to infection, for example, to a virus such as hepatitis virus, or to a bacterium such as *Mycobacterium tuberculosis* (the Gram-positive etiologic agent of tuberculosis) (see Figure 4). Candidate genes are identified and changes in expression of those genes in the presence of a challenge provide a set of markers. The set of markers can combine markers encoded by the genome of the subject and one more distinctive markers encoded by the genome of the infectious agent. For example, changes in expression of an immediate early gene of a virus, e.g. a gene encoding an enzyme of viral replication, and a host gene such as the gene for any or all of IL-2, IL-4 and IL-5, can comprise markers or surrogate markers for a medical condition capable of detecting that condition prior to the onset of medical symptoms. This method affords earlier detection of an infection than is possible using current diagnostic techniques.

(b) Toxicity profiles and mechanistic profiles obtained from an *in vitro* assay and *in vivo* assays. Toxicity and mechanistic information arising from the administration of a compound to a population of cells can be monitored using calibrated profile data sets. The following is an example of an experimental protocol for obtaining this information. Firstly, an experimental groups is established: (1) control cells maintained without therapeutic agent and without stimulus; (2) cells treated with therapeutic agent but without stimulus; (3) cells without therapeutic agent but with stimulus, (4) sample with therapeutic agent and with stimulus. The population of cells can be selected from primary cell cultures prepared in culture plates using methods well established in the art; or mature differentiated cell preparation from whole blood or isolated monocytes from the target organism, which in this example is mouse.

The cells are stimulated so as to present a targeted physiological condition by pretreatment with LPS purified from a Gram-negative bacterium (a variety of LPS preparations from pathogenic bacteria, for example, from *Salmonella typhimurium* and from *Escherichia coli* O1157:H7, are available from Sigma, St. Louis, MO). The therapeutic agent administered to the cell samples in this

example is an inhibitor of an enzyme known to be key in disease etiology, namely an inhibitor of a protease or a nucleic acid polymerase. Following treatment by addition of the therapeutic agent and further incubation for four to six hours, samples of the cells are harvested and analyzed for gene expression. Nucleic acid, specifically RNA, can be prepared from the sample by methods known to one or ordinary skill in the art (see, for example, the Lyse-N-Go™ reagent, Pierce Chem. Co., Rockford, IL). Samples are analyzed by QPCR according to a quantitative replicative procedure, (quantitative polymerase chain reaction procedure (QPCR)) (see, for example, Gibson,U. 1996 Genome Res.6:995-1001, and references cited therein). Total RNA was assessed using universal primers. Toxicity of the agent for cells can be measured in untreated cells by vital stain uptake, rate of DNA synthesis (autoradiography of labeled nucleic compared to cells stained), stain by DNA-specific eyes (Hoechst), etc. Mechanistic profiles can be determined by analysis of the identifies of *de novo* up- or down-regulated genes. Further, in the presence of a therapeutic agent, some genes are not expressed, indicating potential efficacy of the therapeutic agent in suppressing the effects of stimulation by the LPS. For example, in Figure 21, levels of ICE that are somewhat stimulated in the presence of LPS +Echinacea are substantially depressed by LPS + Arnica relative to LPS stimulated cells absent agent. Levels of HSP 70 which are depressed in the presence of LPS+Echinacea are substantially stimulated in the presence of LPS +Arnica, and LPS + Siberian Ginseng relative to LPS stimulated cells absent the addition of an agent. Levels of IL-12p40 which are slightly increased in the presence of LPS + Echinacea are substantially depressed in the presence of LPS + Arnica and LPS +Siberian Ginseng relative to LPS stimulation. In contrast to the above using nutraceuticals, Figure 16 shows a much enhanced reduction of gene expression in whole blood for IL-1a, Il-1b, Il-7, Il-10, IL-IL-15, IFN-g, TGF-b, TNF-b cox-2, and ICAM in the presence of prednisolone +LPS when compared to arnica +LPS or nothing +LPS.

(c) Quantitation of gene expression in a blood cell to predict toxicity in another tissue or organ.

Leukocytes can be obtained from a blood sample of a subject, for the

purpose of assessing the appearance of a pathological condition in another organ, for example, the liver. A profile data set is obtained of genes expressed in the leukocytes, for example, genes encoding a set of lymphokines and cytokines. The data set is compared to that of the database, to examine correlations for example to other subjects, and to the subject prior to administration of a therapeutic agent.

By this method, a correlation can be drawn between, for example, administration of acetaminophen (Tylenol) and sensitivity to this therapeutic agent and manifested by liver damage. An early prediction of therapeutic agent sensitivity, detected prior to the onset of actual damage to the liver, can be clinically available so that the subject receives no further administration of acetaminophen. The success of the database is the ability to detect a correlation or correlations prior to the onset of traditional medical assessments, such as increase in bilirubin level or other indication of liver pathology.

(d) Calibrated profiles from blood cells for prognosis of severity and prediction of adverse reactions in treatment of an autoimmune disease.

The probability and timing of onset of symptoms of an autoimmune disease, for example, rheumatoid arthritis, may be monitored by appearance of expression of markers or surrogate markers as determined by the methods of gene expression profiling of markers or surrogate markers and comparison to a profile database as described above. Thus an indication of imminent onset can be obtained, and advance management by utilization of preventive measures to forestall onset, can be taken. Further, the user can choose a set of potential therapeutic agents, and assess for a given agent, the probability that a subject will present an adverse reaction if given a full course of treatment, prior to that full course. For example, using embodiments of the invention, a single dose of the agent methotrexate can be administered to a subject having arthritis and in need of a therapeutic agent. If the gene expression profile data set of the subject in response to a single dose of methotrexate correlates with data sets from subjects having adverse reactions to this agent, then administration of a full course of methotrexate is counterindicated. Conversely, if the gene expression profile data set correlates with those of subjects who have responded positively to

administration of a course of methotrexate treatment, then this therapeutic agent can be administered to the subject with much lower probability of adverse reaction.

Discussion of Figures

Figures 1-4 illustrate some of the applications of calibrated profile data sets. In Figure 1, three possible scenarios are provided. Firstly, a candidate therapeutic agent may be tested to determine its molecular pharmacology and toxicology profiles. The test might include obtaining calibrated profile data sets for a series of panels selected on the basis of what activity is predicted for the drug. The population of cells exposed to the agent may be the result of in vivo administration as depicted by the mouse or direct exposure in vitro where the cells may be an indicator cell line or an ex vivo sample from the subject. The result of the screen is the identification of more effective drug candidates for testing in human subjects.

The second scenario in Figure 1 is the use of calibrated profile data sets to identify a suitable clinical population for screening a potential therapeutic agent. Both demonstration of lack of toxicity and demonstration of clinical efficacy require certain assumptions about the clinical population. The calibrated profile data sets provides a means for establishing those assumptions with respect to the biological condition of the individuals selected for the clinical trials.

The third scenario in Figure 1 is the opportunity to practice individualized medicine which may include creating an archive of calibrate profile data sets on the individual in a state of health such that changes can be identified using signature panels so as to permit prognosis or diagnosis of a particular condition. Moreover, stored information about the patient in the form of calibrated profile data sets permits selecting one of a group of possible therapeutic agents most like to be effective for the patient, optimizing dosage of drug and detecting adverse effects that might arise through drug interactions before symptoms arise. The result of the use of calibrated profile data sets is to provide more efficient and cost effective health care management.

The novel approach described above for evaluating a biological condition

of a subject may be applied to an ex vivo or in vitro assay for measuring the effect of an agent on a biological condition as illustrated in Figures 2-4. A sample from the patient may measured directly ex vivo or tested ex vivo against an agent to predict an effect in the patient. This provides a quick and effective way to determine which drug chosen from within a single class of drugs that all may be used to treat a particular condition, may be most effective for a given subject. Alternatively, an agent may be tested on an indicator cell line that can provide a quantitative measure of therapeutic performance in a class of individuals.

Figure 2 illustrates how calibrated profile data sets may assist in screening a library of candidate compounds to discover candidate drugs. Starting with for example, 500 candidate drugs, these can be tested in indicator cells or ex vivo body fluid or tissues against signature panels for in vitro toxicology or metabolic indicators. The figure illustrates the large number of compounds that entered in late stages in the development process only to ultimately be rejected due to adverse biological interactions. It is expected that early adoption of the use of calibrated profile data sets will more readily identify likely successful candidates and thereby reduce the expense and untoward effects of animal and human experimentation for compounds that could have been predicted to fail.

Figure 3 describes multiple screens in which a compound might be administered to an experimental animal such as a mouse or to an indicator cell line. The in vivo or ex vivo or indicator cell sample might further be treated with a stimulus. The result of both the compound and the stimulus could then be detected using to signature profiles for toxicity or for mechanism to compare the effect of no drug +/- stimulus or +/-drug and no stimulus. Both in vitro (left panel) and in vivo (right panel) studies can be used to evaluate the effect of a compound (drug, nutraceutical, environmental stimuli, etc.). The right hand panel also illustrates the specific embodiment of an "in vitro clinical trial", that is, treatment of cells obtained from a subject and treated with a compound (with or without a stimulus) in vitro (or ex vivo) in order to predict the outcome of similar treatment of the subject in vivo (see Fig. 15 for a specific example). The output from both panels is described as toxicity and mechanistic profiles. Either

experimental course may be used to both evaluate potential toxicity, e.g., using the toxicity, or liver metabolism panels, and to determine or confirm likely mechanism of action by a critical selection of a gene panel(s) that illustrates and differentiates molecular mechanisms of action (see Figure 12 for a specific example).

Figure 4 illustrates a bioassay in which cells are removed from the subject and tested ex vivo with the addition of a compound and also a challenge or stimulus. The ex vivo effect of stimulus and then drug on whole blood taken from a human subject is shown in Figure 12 in which the stimulus is lipopolysaccharide (an inflammatory agent) while the drug is any of methotrexate, meclofenamate or methylprednisolone using a signature panel for inflammation. Methylprednisolone, a drug commonly used in the treatment of acute exacerbations of COPD as well as in the chronic management of this disease, is considered to be a potent by non-specific anti-inflammatory agent. However, as demonstrated in Figure 22, its effects on gene expression are dependent on the stimulus. While there are general qualitative similarities between the effects on gene expression across these three stimuli, there are both quantitative and qualitative differences that may be important in understanding when glucocorticoid intervention is warranted.

According to embodiments of the invention, an indicator cell population is used to measure quantitative gene expression the effect of an agent or a biological sample may influence the choice of which indicator cell line will be most informative. For example, a cloned cell line such as THP-1 or a primary cell population (peripheral mononuclear cells) may provide information that is comparable to that obtained from a body sample directly (see Figure 15). The normal state of gene expression may range from zero or few transcripts to 10^5 or more transcripts.

Similarly, an agent may be evaluated for its effect on any population of cells, either in vivo, ex vivo or in vitro, by administering the agent and then determining a calibrate profile data set for those cells under the selected conditions. Examples of this approach are provided in Figures 10-16 and 18.

Figure 18 further provides calibrated profile data sets for different concentrations of a single agent showing that the transcription of selected constituents vary with dose and therefore the anticipated effectiveness with respect to the biological condition.

The above description of determining a biological condition is exemplified as follows: The action of a pharmaceutical or nutraceutical is measured with respect to its anti-inflammatory properties. The measurement of the effect may be established using a panel of constituent gene loci for example, an inflammation panel, including, Interleukin 1 alpha (IL-1α) or Tumor Necrosis Factor alpha (TNF-α). The anti-inflammatory effect may first be established by treating indicator cells or sample cells ex vivo with a known inflammation inducers (for example, lipopolysaccharide or other mitogens) followed by treatment with the experimental agent or condition expected to suppress or reduce the expression from the appropriate gene loci. According the baseline profile data set is the delta change in gene expression for a particular panel of constituents. The addition of a potential anti-inflammatory agent results in a second delta change that is superimposed on a first delta change. This is illustrated for example in Figure 12. Methylprednisolone has a substantial down regulation effect on IL-2 in blood cells stimulated ex vivo with LPS where the baseline data set is LPS stimulated cells. In this case there is a negative delta. In contrast, IL-2 appears to be upregulated in whole blood not previously exposed to LPS, where the baseline data set is unstimulated cells. (Figure 16b) This is consistent with the observation that methylprednisolone stimulated IL-2 production.

The determination of the biological condition of a subject may include measuring and storing additional data about the subject. For example, if the subject is a human or mammalian patient, additional clinical indicators may be determined from blood chemistry, urinalysis, X-ray, other chemical assays and physical or sociological findings.

Figure 7 illustrates how the accumulation of calibrated profile data sets can improve the predictive power of the database and thereby increase its value

in generating information about a biological condition or agent. The figure indicates the use of the database in terms of its predictive power to, for example, predict the course of a therapeutic intervention, follow the course of an individual subject compared to a population, prediction of a likely mechanism of metabolism or molecular mechanism of action or a comprehensive database that allows comparison of a single profile to a collection of signature, calibrated precision profiles.

Preferred embodiments of how the database may be used is provided in Figure 8. Figure 8 illustrates display of a data profile set from the source database. Entries for input include a name, an Experimental Type, and whether the entry is a New Reference; Cell/Tissue/Species and whether these are new; Therapeutic agent (compound), Dose, and additional parameters and whether the therapeutic agent is new. Observations are recorded according to the identity of a Gene (New Gene) and a Protein (New Protein). The Stimulus or other Treatment, if any, and the Dose are entered. Gene (and/or Protein) Expression, Expression Value, Expression Units if appropriate and Expression Time are shown. The figure specifically illustrates the range of applicable fields of investigation from complex natural products to clinical trails in humans, linkage to traditional forms of measurement and evaluation such as literature citations, clinical indicators and traditional pharmacokinetic measurements. Expert analysis of the precision profile data contained in the database may then be used to guide product development and marketing, or used to improve the clinical decision making concerning the health of a single individual or population of individuals.

It is anticipated that one form of record might provide information about a subject or agent with respect to identity, medical history including traditional pharmaceutical/medical data, clinical indications as determined from literature data, reference to additional types of analysis in the database, etc.

Figure 9 shows an embodiment of the present invention in which profile data is evaluated using data from a database that is remotely accessed over a network. The figure illustrates that data are expected to be derived at one or

more locations, compared using a central database and information obtained used to affect, for example, the course of treatment of an individual or population. The two-way nature of 1109 illustrates the iterative process whereby the database affects the course of treatment or development, and outcome or response to such intervention again becomes part of the database. In a first location, as in Figure 5, from a tissue sample procured in box 1101, there are derived multiple RNA species pursuant to box 1102, and then in box 1103, profile data are quantified to produce a profile data set that is pertinent to the tissue sample obtained in box 1101. In order to evaluate the profile data set, in box 1104 information is retrieved from database 1108, which is located in a second location. In fact the database may be in communication with a large number of locations, each of which is generating profile data that must be evaluated. The retrieval of information from the database is accomplished over a network 1109, which may include the Internet, in a manner known in the art. Once information has been obtained from the database 1108, the information is used in evaluating the quantified profile data in box 1105, with the result in box 1106 that the medical condition of the subject may be assessed. The database 1108 is in box 1107 updated over the network 1109 to reflect the profile data that have been quantified in box 1103. In this manner the database 1108 may be updated to reflect the profile data obtained over all locations, and each location has the benefit of the data obtained from all of the locations.

EXAMPLES

Example 1.

(a) Use of whole blood for ex vivo assessment of a biological condition affected by an agent

Human blood is obtained by venipuncture and prepared for assay by aliquoting samples for baseline, no stimulus, and stimulus with sufficient volume for at least three time points. Typical stimuli include lipopolysaccharide (LPS), phytohemagglutinin (PHA) and heat-killed staphylococci (HKS) or carrageean and may be used individually (typically) or in combination. The aliquots of

heparinized, whole blood are mixed without stimulus and held at 37°C in an atmosphere of 5% CO2 for 30 minutes. Stimulus is added at varying concentrations, mixed and held loosely capped at 37°C for 30 min. Additional test compounds may be added at this point and held for varying times depending on the expected pharmacokinetics of the test compound. At defined times, cells are collected by centrifugation, the plasma removed and RNA extracted by various standard means.

(b) Preparation of RNA for measuring gene expression

Nucleic acids, RNA and or DNA are purified from cells, tissues or fluids of the test population or indicator cell lines. RNA is preferentially obtained from the nucleic acid mix using a variety of standard procedures (or RNA Isolation Strategies, pp.55-104, in RNA Methodologies, A laboratory guide for isolation and characterization, 2nd edition, 1998,Robert E. Farrell, Jr., Ed., Academic Press); in the present use using a filter-based RNA isolation system from Ambion (RNAqueous ™, Phenol-free Total RNA Isolation Kit, Catalog #1912, version 9908; Austin, Texas). Specific RNAs are amplified using message specific primers or random primers. The specific primers are synthesized from data obtained from public databases (e.g., Unigene, National Center for Biotechnology Information, National Library of Medicine, Bethesda, MD), including information from genomic and cDNA libraries obtained from humans and other animals. Primers are chosen to preferentially amplify from specific RNAs obtained from the test or indicator samples, see, for example, RT PCR, Chapter 15 in RNA Methodologies, A laboratory guide for isolation and characterization, 2nd edition, 1998,Robert E. Farrell, Jr., Ed., Academic Press; or Chapter 22 pp.143-151, RNA isolation and characterization protocols, Methods in molecular biology, Volume 86, 1998, R. Rapley and D. L. Manning Eds., Human Press, or 14 in Statistical refinement of primer design parameters, Chapter 5, pp.55-72, PCR applications: protocols for functional genomics, M.A.Innis, D.H. Gelfand and J.J. Sninsky, Eds., 1999, Academic Press). Amplifications are carried out in either isothermic conditions or using a thermal cycler (for example, a ABI 9600 or 9700 or 7700 obtained from PE Biosystems, Foster City, CA; see Nucleic acid detection

methods, pp. 1-24, in Molecular methods for virus detection, D.L.Wiedbrauk and D.H., Farkas, Eds., 1995, Academic Press). Amplified nucleic acids are detected using fluorescent-tagged detection primers (see, for example, Taqman™ PCR Reagent Kit, Protocol, part number 402823 revision A, 1996, PE Applied Biosystems, Foster City CA.) that are identified and synthesized from publicly known databases as described for the amplification primers. In the present case, amplified DNA is detected and quantified using the ABI Prism 7700 Sequence Detection System obtained from PE Biosystems (Foster City, CA). Amounts of specific RNAs contained in the test sample or obtained from the indicator cell lines can be related to the relative quantity of fluorescence observed (see for example, Advances in quantitative PCR technology: 5' nuclease assays, Y.S. Lie and C.J. Petropolus, Current Opinion in Biotechnology, 1998, 9:43-48, or Rapid thermal cycling and PCR kinetics, pp. 211-229, chapter 14 *in* PCR applications: protocols for functional genomics, M.A.Innis, D.H. Gelfand and J.J. Sninsky, Eds., 1999, Academic Press.

Example 2. Different inflammatory stimuli give rise to different, baseline profile data sets so that the calibrated precision profiles for different agents in the same class of anti-inflammatory result in different signature profiles.

Figure 11 documents the usefulness of different inflammatory stimuli to give rise to different, baseline profile data sets so that the calibrated precision profile data sets for the three anti-inflammatory agents tested result in different signature profiles. The different profiles reflect the difference in the molecular targets and mechanisms of action of the three agents derived from a single class of therapeutics, anti-inflammatory agents. The figure also illustrates the extraordinary range of detection (y-axis) from less than 10 fold difference from the calibrated profile to plus or minus 10E13 increase or decrease in gene expression when compared to the calibrator. Comparison to the calibrator results in gene expression profiles that are increased, decreased, or without change from the calibrated set.

Figure 11(a) shows relative gene expression (mRNA synthesis) in heat-killed staphylococci (HKS)-stimulated cells, and the effect of three different compounds

(TPCK, UT-77, and "Dex", or dexamethasone). Compound TPCK caused a 10-fold decrease in relative IFN-γ expression, and 100,000-fold decreases in IL-4 and IL-5 expression. Further, compound UT-77 caused even greater magnitude of increases in relative expression of the gene encoding IL-5, and more modest increases in IL-1 expression (more than 10-fold) and IFN-γ Such effects can be highly significant in disease etiologies and outcomes, and have predictive value concerning the usefulness as therapeutic agents of these compounds or similar chemical entities or chemicals that act similarly. HKS cells are an *in vitro* model of Gram-positive bacterial infection.

Fig. 11(b) displays analyses of expression of the 12 genes in lipopolysaccharide-(LPS)-treated cells, an *in vitro* model of Gram-negative bacterial infection. These data include several striking contrasts to the data in Fig. 11(a). Thus treatment with the therapeutic agent Dex caused a striking decrease in expression of the IL-2 gene in LPS-treated cells, and a striking increase in IL-2 expression in HKS-treated cells. Strikingly large differences in gene expression in the differently stimulated cells can be seen for the IL-4 and the IL-5 genes. Expression of the gene for IFN, in contrast, responded similarly in cells treated by either of the stimuli and any of the therapeutic agents.

By these criteria, expression of the genes for IL-2, IL-4 and IL-5 were observed to be candidate markers or surrogate markers in cell model systems to distinguish responses of the cells to Gram-positive and Gram-negative bacterial infection.

Example 3. A single therapeutic agent for treating a particular condition can be differentiated from a second therapeutic agent that also treats the particular condition by a signature profile for a given panel of gene loci.

Figure 12 shows a calibrated profile data set for a panel having 8 constituents that are indicative of a biological condition that includes inflammation. The profiles are shown for three different anti-inflammatory agents-methotrexate, meclofenamate and methylprednisolone. The calibrated profile data sets for each agent as shown represents a signature profile for that agent. This signature profile may serve as a device for establishing quality

control for a batch of the agent. Indeed, it is envisaged that compounds or classes of compounds on the market or in development may be characterized by a signature profile. The signature profile may be represented in a graphical format, more particularly as a bar graph as provided in Figure 12. For Figure 12, an ex vivo sample was tested. A sample of blood was taken from the subject. Aliquots of the sample were subjected to lipopolysaccharide (LPS) ex vivo. After 30 minutes, the anti-inflammatory agent as indicated was added to an aliquot of the sample of blood and after about another 4 hours, the expression of the panel of genes (Il-1a, Il-2, Il-8, Il-10, Il-12p35, Il-12p40, IL-15, IFN-Gamma and TNF-a) was determined. Although the calibrated profile of methotrexate and meclofenamate were similar, the calibrated profile of methylprednisolone was substantially different. Differences may be reflective of the differences of the mechanisms or target(s) of action of this agent within the general class of anti-inflammatory compounds. The baseline is the profile data set for lipopolysaccharide absent any additional agents.

Example 4. There is relatively low variability with respect to the profile within a single individual over time when the calibrated precision profile is determined from the measurement of gene expression across many gene loci that have been appropriately induced.

Figure 13(a)(b) and (c) show a graphical representation of calibrated precision profile data sets for two different samples of whole blood. Heparinized whole blood from a single normal healthy volunteer was collected on two separate occasions of more than 2 weeks apart. Figure 13a for sample 991116 and Figure 13b,for sample 991028 reflect the biological condition of the tested cells from the single donor using a panel (i.e., the inflammation panel) of 24 members, in response to stimulation with one of three different agents. . The baseline in this example is derived from untreated cells obtained from the same individual. The calibrated profiles are shown for cells exposed for 4 to 6 hours to lipopolysaccharide (LPS), heat-killed Stapylococci (HKS), and phytohemagglutinin (PHA). Figure 13c shows a direct comparison of LPS-stimulated blood sample 99116 with respect to blood sample 991028, i.e., 991028

is used as the calibrator or baseline profile data set. The messenger RNA levels measured on 10/28/99 were used to compare the levels of messenger RNA measured on 11/16/99. A perfect identity of RNA levels would be represented by a flat line at unity. These data clearly show that for baseline gene expression, there can be as much as an 8 fold difference (c-jun) in messenger RNA levels. However, for most of the genes measured, the levels of messenger RNA measured on one day are within 2-3 fold of those measured on a different day. !3 (d) is similar to 13(c) except that the cells were not stimulated with LPS.

The figure documents the relatively low variability with respect to the profile within a single individual over time when the calibrated precision profile is determined from the measurement of gene expression across many gene loci that have been appropriately induced. The figure illustrates (1) the class-specific effects (generally inflammatory as determined by the effect on pro-inflammatory gene loci, e.g. TNF-alpha, IL-1 alpha and IL-1 beta), (2) the agent-specific effects quantitative differences between each of the agents at the same gene loci (e.g., IL-2) and (3) reproducible and therefore predictable effects on the subject population, TK (Figure 13c)

Example 5. Similarities and differences in the effect of a single agent on cell populations differing in their biological condition.

Ex-vivo gene expression analysis can be performed by obtaining the blood of a subject for example by drawing the blood into a vacutainer tube with sodium heparin as an anticoagulant. An anti-inflammatory such as 3-methyl-prednisolone at a final concentration of 10 micromolar was added to blood in a polypropylene tube, incubated for 30 minutes at 37C. in 5% CO_2 . After 30 minutes a stimuli such as LPS at 10 ng/mL or heat killed staphlococcus (HKS) at 1:100 dilution was added to the drug treated whole blood. Incubation continued at 37C. in 5% CO_2 for 6 hours unless otherwise indicated. Erythrocytes were lysed in RBC lysis solution (Ambion) and remaining cells were lysed according to the Ambion RNAqueous-Blood module (catalog # 1913). RNA was eluted in Ambion elution solution. RNA was DNAsed treated with 1 unit of DNAse I

(Ambion #2222) in 1X DNAse buffer at 37C. for 30 minutes. First strand synthesis was performed using the Perkin-Elmer TaqMan Reverse Transcriptase kit with MultiScribe reverse transcriptase (catalog # N808-0234). Quality check of RT reactions were performed with Taqman PCR chemistry using the 18S rRNA pre-developed assay reagents (PDAR) from PE Biosystems (part #4310893E). PCR assay of Source Precision Profiles were performed on 6 to 24 genes in four replicates on the PE Biosystems 7700. PCR assays were performed according to specifications outlined with the PDAR product. Relative quantitation of the gene of interest was calibrated against 18S rRNA expression as described in PE product User Bulletin 2 (1997) and elaborated in Hirayama, et al (Blood 92, 1998:46-52) using 18S instead of GAPDH.

Relative quantitation of the mRNA was measured by the difference in threshold cycles between 18S and the gene of interest. This delta C_T was then compared to the normalizing condition, either subject before treatment, or stimuli without drug in an ex-vivo assay to measure "fold induction" represented in the bar graphs. (Figure 14) For example in the above graph, IFN-levels are 1/50 less on day 3 than before treatment.

Example 6.In Vivo and Ex vivo samples provide comparable signature profiles

Figure 15 shows the calibrated profile data set for two subjects (Subject 1 and Subject 2) who have been treated over a three day period with a standard dose of the corticosteroids, dexamethasone. Blood from each subjects was obtained 72 hours later and a quantitative measure of the amount of RNA corresponding to the panel constituents was determined. Although, the calibrated profile data set for each subject was similar for most gene loci, some notable differences were also detected, for example for Il-2, Il-10, Il-6 and GM-CSF. A calibrated profile data set is also shown for comparison for an ex vivo sample of blood from sample 1 prior to treatment with corticosteroid where the ex vivo sample is subjected to an equivalent amount of corticosteroid in vitro as calculated to be the plasma level in the subject. The similarity in the calibrated profile data set for ex vivo samples when compared to in vivo samples provides support for an in vitro assay that will predict the in vivo action of the compound.

We have observed a similar comparable effect between in vivo and ex vivo samples infected with an infectious agent, more particularly bacterial or viral agents. We have concluded therefore that the ex vivo samples provide an effective method of determining the effect of a single compound or multiple compounds on a patient, where the multiple compounds may be either used in combination, in parallel or sequentially to optimize the selection of an agent for a biological condition for the subject.

Example 7. Demonstration of reproducibility of an in vitro response with an approved anti-inflammatory on 5 different donor subjects.

Comparison and analysis of the Figures 18a through 18e demonstrates the consistency of effect of the stimulus and in vitro treatment with an approved anti-inflammatory on 5 different donors (each figure representing a unique donor). The use of a known and tested stimulus results in a highly reproducible gene response in vitro that may be correlated with a predictable in vivo response. Figures 18a-18e provide the results of analysis of 5 donors from which a blood sample has been taken. The blood samples were exposed to a therapeutic agent at various concentrations ranging from 0.1μM to 5μM, more particularly 0.1μM, 0.3μM, 1μM, 3μM and 5μM, for a 4 hour period. Different concentrations of the drug resulted in a calibrated profile data set for an inflammation panel at each concentration that was qualitatively different from the next. Figure 18a corresponds to donor 1, Figure 18b corresponds to donor 2, Figure 18c corresponds to donor 3, Figure 18d corresponds to donor 4 and Figure 18e corresponds to donor 5. Each individual varied from the other and also provided a variable profile for a different concentration. This set of figures illustrates the high level of information obtainable by calibrated profile data sets.

Example 8. A calibrated profile data set may provide a signature profile for a complex mixture of compounds

Figure 21 illustrates the effect of three different anti-inflammatory herbs on a panel of constituents including constituents of an inflammatory panel (TNF-a, Il-1b, ICAM, Il-8, Il-10, Il-12p40, ICE, cox-2, cox-1 and mmp-3) a cell growth and differentiation panel (c-fos, c-jun and STAT3) , a toxicity panel (SOD-1, TACE, GR,

HSP70, GST, c-fos, c-jun, INOS) and a liver metabolism panel (INOS, cyp-a and u-pa). The cells assayed in Figure 21 are aliquots of blood from a subject that are exposed ex vivo to lipopolysaccharide and to Echinacea (SPM9910214) Arnica (SPM9910076) and Siberian Ginseng (SPM9910074), each of the nutraceuticals being applied to the blood sample at the same concentration of 200ug/ml. The baseline is cell sample with lipopolysaccharide in the absence of a nutraceutical. Each nutraceutical (formed from a complex mixture)has a characteristic signature profile just as did the single compound pharmaceutical anti-inflammatory agents. The signature profile may be provided in a graphic form that can be use to identify a herbal while providing information concerning its properties and its efficacy for a single subject or for an average population of subjects.

Example 9. A quality control assay for Echinacea brands using calibrated profile data sets

Figure 24 shows a graphic representation of the calibrated profile data sets for four different commercial brands of Echinacea. Brands using an Inflammation Panel. As expected, SPM007 and SPM003 gave the signature, calibrated profiles similar to authentic Echinacea. Samples SPM010 and SPM 016, although labeled and sold as Echinacea when tested using the system described in Figure 14, resulted in signature calibrated profiles that were substantially similar to the profile obtained with lipopolysaccharide alone. Echinacea samples SPM010 and SPM016 were found to have elevated, highly biologically active levels of endotoxin while the LPS levels in SP007 and SP003 were undetectable. A stored signature profile for active echinacea obtained from a panel designed to test efficacy and mode of action, e.g., the inflammation panel, permits evaluation of new batches of Echinacea, differentiation of existing or new brands of Echinacea, guide the isolation and development of new compounds with different or similar activities from a complex compound like Echinacea or may be used in the development of quality assurance in the production, analysis and sale of new or previously marketed compounds. In the example cited, two of the brands of Echinacea SP010 and SP016.result in calibrated profiles that are characteristic of authentic Echinacea.

Example 10. Comparison of three herbal preparations using an indicator cell line

Figures 25 (a) –(c) provide calibrated profile data sets for three herbal preparations with respect to an indicator cell line (THP-1) rather than a blood sample from a subject. In Figure 25(a), the baseline is the profile data set for THP-1 cells absent the herbal while the histograms represent the calibrated profile data sets for the same herbal from three different manufacturing sources of the same herb at 250ug/ml. Gene expression results are shown on a log scale. Similar to the observation in Figure 14, these demonstrate that similarly labeled compounds obtained from different sources have demonstrable and quantifiable differences in calibrated profiles using a specific panel, eg. The inflammation panel designed to obtain information about the expression of gene products realted to inflammation and infection. This suggests that the compounds likely have different efficacies when used for specific purposes.

Figure 25(b) provides a comparison of the calibrated profile of a single herb at three concentrations using the indicator cell line of THP-1. The baseline profile data set is untreated THP-1 cells. Analysis of the data suggests a concentration-dependent response in the indicator cell lines which, although demonstrated here, may be indicative of a similar response in subjects.

Figure 25(c) provides a comparison of four commercial echinacea brands used at the same concentration and tested against a panel of constituents using a THP-1 cell line as an indicator cell population. Differential expression, as revealed by differences in the calibrated profiles, allows direct comparisons of complex compounds to be made. For example, analysis of the differences in the calibrated profiles could be used to guide compound isolation and development, product differentiation in the marketplace, or used by the consumer or health professional to guide the individualized choice of a single compound from a class of similar compounds that may be suited for a particular biological condition.

Although various embodiments of the invention have been disclosed, it should be apparent to those skilled in the art that various changes and modifications can be made which will achieve some of the advantages of the invention without departing from the true scope of the invention. These and other obvious modifications are intended to be covered by the appended claims.

What is claimed is:

1. A method, for evaluating a biological condition of a subject, comprising:

a. obtaining from the subject a sample having at least one of RNAs and proteins;

b. deriving from the sample a first profile data set, the first profile dataset including a plurality of members, each member being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of the biological condition; and

c. producing a calibrated profile data set for the panel, wherein each member of the calibrated profile data set is a function of a corresponding member of the first profile data set and a corresponding member of a baseline profile data set for the panel, the calibrated profile data set providing a measure of the biological condition of the subject.

2. A method, for evaluating a biological condition of a subject, comprising:

a. obtaining from the subject a first sample having at least one of fluid, cells and active agents;

b. applying the first sample or a portion thereof to a defined population of indicator cells;

c. obtaining from the indicator cells a second sample containing at least one of RNAs or proteins;

d. deriving from the second sample a first profile data set, the first profile data set including a plurality of members, each member being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of the biological condition; and

e. producing a calibrated profile data set for the panel, wherein

each member of the calibrated profile data set is a function of a corresponding member of the first profile data set and a corresponding member of a baseline profile data set for the panel, the calibrated profile data set providing a measure of the biological condition of the subject.

3. A method, for evaluating a biological condition affected by an agent, the method comprising:

a. obtaining, from a target population of cells to which the agent has been administered, a sample having at least one of RNAs and proteins;

b. deriving from the sample a first profile data set, the first profile data set including a plurality of members, each member being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of the biological condition; and

c. producing a calibrated profile data set for the panel, wherein each member of the calibrated profile data set is a function of a corresponding member of the first profile data set and a corresponding member of a baseline profile data set for the panel, the calibrated profile data set providing a measure of the biological condition as affected by the agent.

4. A method according to any of claims 1 through 2, wherein the baseline profile data set is derived from one or more other samples from the same subject taken under conditions different from those of the sample.

5. A method according to claim 4, wherein the conditions are selected from the group consisting of (i) the time at which a given sample is taken, (ii) the site from which a given sample is taken, (iii) the physiological condition of the subject when a given sample is taken.

6. A method according to claim 4, wherein the one or more other samples are taken over an interval of time that is at least twelve months between

an initial sample and the sample.

7. A method according to claim 4, wherein the one or more other samples are taken over an interval of time that is at least one month between an initial sample and the sample.

8. A method according to any of claims 1 through 3, wherein the sample is derived from blood and the baseline profile data set is derived from tissue or body fluid of the subject other than blood.

9. A method according to claim 4, wherein the baseline profile data set is derived from one or more other samples from the same subject, taken when the subject is in a physiological condition different from that in which the subject was at the time the sample was taken, with respect to at least one of age, diet, medication, and environmental exposure.

10. A method according to claim 3, wherein the baseline profile data set is derived from one or more other samples from the same population taken under conditions different from those of the sample.

11. A method according to claim 10, wherein the conditions are selected from the group consisting of (i) the time at which a given sample is taken and (ii) the physiological condition of the population when a given sample is taken.

12. A method according to claim 10, wherein the one or more other samples are taken over an interval of time that is at least twelve months between an initial sample and the sample.

13. A method according to claim 10, wherein the one or more other samples are taken over an interval of time that is at least one month between an

initial sample and the sample.

14. A method according to claim 10, wherein the sample is derived from blood and the baseline profile data set is derived from tissue or body fluid of the subject other than blood.

15. A method according to claim 10, wherein the baseline profile data set is derived from one or more other samples of cell populations associated with a common subject, the populations taken when the subject is in a physiological condition different from that in which the subject was at the time the sample was taken, with respect to at least one of age, diet, medication, and environmental exposure.

16. A method according to any of claims 1 and 2, wherein the baseline profile data set is derived from one or more other samples from one or more different subjects.

17. A method according to claim 16, wherein the one or more different subjects have in common with the subject at least one of age group, gender, ethnicity, geographic location, diet, medical disorder, clinical indicator, medication, physical activity, body mass, and environmental exposure.

18. A method according to claim 3, wherein the baseline profile data set is derived from one or more other samples from one or more cell populations associated with different subjects.

19. A method according to claim 18, wherein the one or more different subjects have in common with the subject at least one of age group, gender, ethnicity, geographic location, diet, medical disorder, clinical indicator, medication, physical activity, body mass, and environmental exposure.

20. A method according to any of claims 1 through 3, further comprising: interpreting the calibrated profile data set in the context of at least one other clinical indicator.

21. A method according to claim 20, wherein the indicator is selected from the group consisting of blood chemistry, urinalysis, X-ray, other chemical assays, and physical findings.

22. A method according to any of claims 1 through 3, wherein the biological condition is a complex disease process, involving multiple genes, the disease being of a type involving at least one of inflammation, auto-immune disease, degenerative disease, allergy, vascular disease, ischemia, cancer, developmental disease, hormonal condition, aging and infectious diseases.

23. A method according to claim 22, wherein the biological condition is one of arthritis, asthma, multiple sclerosis, and perimenopausal change.

24. A method according to any of claims 1 and 2, wherein the subject is a living organism.

25. A method according to claim 24, wherein the subject is a mammal.

26. A method according to claim 3, wherein the population of cells is human cells.

27. A method according to claim 3, wherein the population of cells is mammalian cells.

28. A method according to any of claims 1 through 3, wherein the sample is derived from one or more of body fluid and tissue.

29. A method according to any of claims 1 through 3, wherein the sample is derived from blood.

30. A method according to any of claims 1 through 3, wherein the sample is derived from one of a biopsy, a needle aspirate, a lavage specimen, a scraping, and a surgical specimen.

31. A method according to any of claims 1 through 3, wherein the sample is derived from tissue or fluid of a type distinct from that with respect to which the condition is clinically manifested.

32. A method according to any of claims 1 through 3, wherein the condition is a disease and the sample is derived from tissue or fluid of a type distinct from that which is a primary target of the disease.

33. A method according to any of claims 1 through 3, wherein the function is other than a simple difference.

34. A method according to claim 33, wherein the function is a second function of the ratio of the corresponding member of first profile data set to the corresponding member of the baseline profile data set.

35. A method according to claim 34, wherein the function is a logarithmic function.

36. A method according to any of claims 1 through 3, wherein each member of the calibrated profile data set is reproducible with respect to similar samples taken from the subject under similar conditions.

37. A method according to any of claims 1 through 3, wherein each member of the calibrated profile data set is reproducible within one order of

magnitude with respect to similar samples taken from the subject under similar conditions.

38. A method according to any of claims 1 through 3, wherein each member of the calibrated profile data set is reproducible within fifty percent with respect to similar samples taken from the subject under similar conditions.

39. A method according to any of claims 1 through 3, wherein each member of the calibrated profile data set is reproducible within twenty percent with respect to similar samples taken from the subject under similar conditions.

40. A method according to claim 34, wherein each member of the calibrated profile data set is reproducible within one order of magnitude with respect to similar samples taken from the subject under similar conditions.

41. A method according to claim 34, wherein each member of the calibrated profile data set is reproducible within fifty percent with respect to similar samples taken from the subject under similar conditions.

42. A method according to claim 34, wherein each member of the calibrated profile data set is reproducible within twenty percent with respect to similar samples taken from the subject under similar conditions.

43. A method according to claim 34, wherein each member of the calibrated profile data set has biological significance if it has a value differing by more than an amount D, where D= F(1.1) – F(.9), and F is the second function.

44. A method according to any of claims 1 through 3, wherein the biological condition concerns an organ and the panel of constituents enables measurement of the condition in relation to the organ.

45. A method according to any of claims 1 and 2, wherein the biological condition concerns a system of the subject, the system selected from the group consisting of respiratory, vascular, nervous, metabolic, urinary, reproductive, structural, and immunological systems, and the panel of constituents enables measurement of the condition of the subject in relation to the system.

46. A method according to claim 3, wherein the population of cells is derived from a subject and the biological condition concerns a system of the subject, the system selected from the group consisting of respiratory, vascular, nervous, metabolic, urinary, reproductive, structural, and immunological systems, and the panel of constituents enables measurement of the condition of the subject in relation to the system.

47. A method according to claim 46 and the panel includes at least half of the constituents of the Inflammation Panel.

48. A method according to claim 46 and the panel includes at least eighty percent of the constituents of the Inflammation Panel.

49. A method according to claim 46 and the panel includes at least half of the constituents of the Cell Growth and Differentiation Panel.

50. A method according to claim 46 and the panel includes at least eighty percent of the constituents of the Cell Growth and Differentiation Panel.

51. A method according to claim 46 and the panel includes at least half of the constituents of a Toxicity Panel.

52. A method according to claim 46 and the panel includes at least eighty percent of the constituents of a Toxicity Panel.

53. A method according to any of claims 1 through 3, wherein the number of constituents in the panel is at least three but less than 100.

54. A method according to any of claims 1 through 3, wherein the number of constituents in the panel is at least four but less than 100.

55. A method according to any of claims 1 through 3, wherein the number of constituents in the panel is at least at least five but less than 100.

56. A method according to any of claims 1 through 3, wherein the number of constituents in the panel is at least is at least six.

57. A method according to claim 3, wherein the agent is selected from the group consisting of a drug, a mixture of compounds, a functional food, a nutraceutical, a therapeutic agent, an allergen, and a toxin.

58. A method according to any of claims 1 through 3, wherein deriving the first profile data set from the sample includes hybridizing the sample with a set of nucleic acid probes.

59. A method according to claim 58, wherein the probes are attached to an insoluble matrix and the sample is applied to the matrix.

60. A method according to claim 3, wherein evaluating the condition affected by the agent includes evaluating the interaction of the agent with a second agent administered to the population of cells.

61. A method according to claim 60, wherein the interaction is neutral.

62. A method according to claim 60, wherein the interaction is interference.

63. A method according to claim 60, wherein the interaction is cumulative.

64. A method according to claim 60, wherein the interaction is synergistic.

65. A method according to claim 60, wherein the agent is a pharmaceutical.

66. A method, for evaluating the effect on a biological condition by a first agent in relation to the effect by a second agent, the method comprising:

a. obtaining, from first and second target populations of cells to which the first and second agents have been respectively administered, first and second samples respectively, each sample having at least one of RNAs and proteins;

b. deriving from the first sample a first profile data set and from the second sample a second profile data set, the profile data sets each including a plurality of members, each member being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of the biological condition; and

c. producing for the panel a first calibrated profile data set and a second profile data set, wherein (i) each member of the first calibrated profile data set is a function of a corresponding member of the first profile data set and a corresponding member of a first baseline profile data set for the panel, and (ii) each member of the second calibrated profile data set is a function of a corresponding member of the second profile data set and a corresponding member of a second baseline profile data set for the panel, the calibrated profile data sets providing a measure of the effect by the first agent on the biological condition in relation to the effect by the second agent.

67. A method according to claim 66, wherein the first agent is a drug and the second agent is a complex mixture.

68. A method according to claim 66, wherein the first agent is a drug and the second agent is a nutriceutical.

69. A method according to any of claims 1 through 3, wherein obtaining the sample and quantifying the first profile data set are performed at a first location, and producing the calibrated profile data set includes using a network to access a database stored on a digital storage medium in a second location.

70. A method according to claim 69, further comprising updating the database to reflect the first profile data set quantified from the sample.

71. A method according to claim 69, wherein using a network includes accessing a global computer network.

72. A method of conducting a clinical trial of an agent, the method comprising:

a. causing the blind administration of a selected one of a placebo and the agent to each candidate of a pool of subjects; and

b. using quantitative gene expression to monitor an effect of such administration.

73. A method according to claim 72, wherein the pool of subjects is selected using quantitative gene expression analysis on a plurality of candidates to identify those candidates likely to show a response to the agent.

74. A method according to claim 72, wherein the administration

includes determining at least one of a dosage and a dosage range by using quantitative gene expression analysis.

75. A method according to claim 72, further comprising using quantitative gene expression analysis to assist in determining at least one of efficacy and toxicity of the agent.

76. A method according to any of claims 72 through 75, wherein using quantitative gene expression analysis includes using the method of at least one of claims 1, 2, and 3.

77. A digital storage medium on which is stored a computer readable calibrated profile data set, wherein:

a. the calibrated profile data set relates to a sample having at least one of RNAs and proteins derived from a target cell population to which an agent has been administered; and

b. the calibrated profile data set includes a first plurality of members, each member being a quantitative measure of a change in an amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of a biological condition as affected by administration of the agent.

78. A digital storage medium according to claim 77, wherein: (i) each member of the calibrated profile data set is a function of a corresponding member of a post-administration data set and a corresponding member of a baseline data set; (ii) each member of the baseline data set is a quantitative measure of the amount of a distinct RNA or protein constituent in the panel under a normative condition; and (iii) each member of the post-administration data set is a quantitative measure of the amount of a distinct RNA or protein constituent in the panel under a condition following administration of the agent to the target cell population.

79. A medium according to claim 78, wherein the function is a second function of the ratio of the corresponding member of baseline data set to the corresponding member of the post-administration data set.

80. A medium according to claim 79, wherein the second function is a logarithmic function.

81. A digital storage medium according to any of claims 77-80, wherein the agent is a pharmaceutical.

82. A digital storage medium according to any of claims 77-80, wherein the agent includes a second plurality of components.

83. A digital storage medium according to any of claims 77-80, wherein the agent is a nutraceutical.

84. A digital storage medium according to any of claims 77-80, wherein the first plurality is at least three but less than 1000.

85. A digital storage medium according to any of claims 77-80, wherein the first plurality is at least four but less than 1000.

86. A digital storage medium according to any of claims 77-80, wherein the first plurality is at least five but less than 1000.

87. A digital storage medium according to any of claims 77-80, wherein the first plurality is at least six.

88. A digital storage medium on which is stored a plurality of records R_i relating to a population of subjects, each record R_i corresponding to a distinct

instance P_i of a computer readable profile data set P wherein:

a. each instance P_i of the profile data set P relates to a distinct sample derived from a subject, the sample having at least one of RNAs and proteins;

b. the profile data P set includes a plurality of members M_j, each member M_j being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of a biological condition;

~~c. each record R_i includes, for each member M_{ij} of a~~ corresponding distinct instance P_i of the profile data set P, a value corresponding to the value of the member M_{ij}; and

d. each record R_i also includes a reference to a characteristic of the subject relative to the record, the characteristic being at least one of age group, gender, ethnicity, geographic location, diet, medical disorder, clinical indicator, medication, physical activity, body mass, and environmental exposure.

89. A digital storage medium according to claim 88, wherein each sample is derived from a target cell population to which has been administered an agent, such target cell population being derived from a subject.

90. A digital storage medium on is stored a large number of computer readable profile data sets, wherein:

a. each profile data set relates to a sample derived from a target cell population to which has been administered an agent, the sample having at least one of RNAs and proteins;

b. each profile data set includes a plurality of members, each member being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of a biological condition; and

c. the panel is the same for all profile data sets.

91. A method, for evaluating a biological condition of a subject, based on a sample from the subject, the sample having at least one of RNAs and proteins, the method comprising:

a. deriving from the sample a first instance of a profile data set, the profile data set including a plurality of members, each member being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of the biological condition; and

b. producing a first instance of a calibrated profile data set for the panel, wherein each member of an instance of the calibrated profile data set is a function of a corresponding member of an instance of the profile data set and a corresponding member of an instance of a baseline profile data set for the panel, the calibrated profile data set providing a measure of the biological condition of the subject; and

c. accessing a data in a condition database, the condition database having a plurality of records relating to a population of subjects, each record corresponding to a distinct instance of the calibrated profile data set; and

d. evaluating the first instance of the calibrated profile data set in relation to data in the condition database.

92. A method according to claim 91, wherein accessing the condition database includes accessing the condition database over a network.

93. A method according to claim 92, wherein the network is a global computer network.

94. A method according to claim 92, further comprising supplementing the condition database based on data associated with the first instance of the calibrated profile data set.

95. A method according to claim 92, wherein the biological condition concerns a system of the subject, the system selected from the group consisting of

respiratory, vascular, nervous, metabolic, urinary, reproductive, structural, and immunological systems and the panel of constituents enables measurement of the condition of the subject in relation to the system.

96. A method according to claim 92, wherein each record also references a characteristic of the population relative to the record, the characteristic being at least one of age group, gender, ethnicity, geographic location, diet, medical disorder, clinical indicator, medication, physical activity, body mass, and environmental exposure.

97. A method according to claim 96, wherein the characteristic includes a clinical indicator.

98. A method of displaying quantitative gene expression analysis data associated with measurement of a biological condition, the method comprising:

a. identifying a first profile data set pertinent to the gene expression analysis data, the first profile data set including a plurality of members, each member being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of the biological condition;

b. producing a calibrated profile data set for the panel, wherein each member of the calibrated profile data set is a function of a corresponding member of the first profile data set and a corresponding member of a baseline profile data set for the panel, the calibrated profile data set providing a measure of the biological condition of the subject; and

c. displaying the calibrated profile data set in a graphical format.

99. A method according to claim 98, wherein the function is a second function of the ratio of the corresponding member of first profile data set to the

corresponding member of the baseline profile data set.

100. A method according to claim 97, wherein the function is a logarithmic function.

101. A method according to claim 97, wherein the graphical format is a bar graph for each member of the calibrated profile data set.

102. A descriptive record of a change in a biological condition in a population of cells, comprising:

a. a first set of numerical gene expression values for a panel of gene loci, each value in the set corresponding to a single gene locus in a panel of gene loci, the set of values forming a profile data set for a population of cells subjected to a first biological condition;

b. a second set of numerical gene expression values for the panel of gene loci, each value in the set corresponding to a single gene locus, the set of values forming a baseline profile data set for a second population of cells subjected to a second biological condition, the second set of values optionally being an average for multiple gene expression values from multiple populations of cells for each locus in the panel; and

c. a third set of numbers corresponding to the ratio of the first set of values and the second set of values with respect to each gene locus in the panel, the third set being a calibrated profile data set; the profile data set and the calibrated profile data set being descriptive of the first biological condition with respect to the second biological condition.

103. A record according to claim 102, wherein the first population of cells and the second or more population of cells are the same population of cells.

104. A record according to claim 102, wherein the first population of cells and the second or more population of cells are different populations of cells.

105. A descriptive record, according to claim 102, wherein a sample is obtained from a subject, for subjecting the cells to a biological condition, the cell population being an indicator cell population.

106. A gene expression profile data set, according to claim 102, wherein the population of cells is in a subject or derived from a subject.

~~107. A method for diagnosing a biological condition of a subject,~~ comprising :

obtaining a sample from a subject; subjecting a population of cells to the sample and determining the presence of a first biological condition with respect to a second biological condition according to any of claims 1 through 3.

108. A method according to claim 107, further comprising: selecting the subject for a clinical trial according to the biological condition of the subject, so as to determine predictively whether the subject will respond to a test compound if the compound has a predetermined biological activity.

109. A method according to claim 108, wherein the test compound is a pharmaceutical agent.

110. A method according to claim 108 where the test compound is a nutraceutical agent.

111. A method for diagnosing a susceptibility for a biological condition in a subject, comprising:

a. obtaining a sample from the subject;

b. creating a descriptive record, according to any of claims 102 through 106, wherein the set of baseline values is an average of second values contained in a library of baseline profile data sets for the second biological condition; the library containing a plurality of baseline profile data sets grouped

according to a predetermined biological condition; and

c. diagnosing the susceptibility of the subject.

112. A method for monitoring the progress of a biological condition, comprising:

a. creating a plurality of descriptive records, according to any ~~of claims 102 through 106, wherein each set of first values is determined at~~ preselected time intervals with respect to each of the other gene expression profiles;

b. comparing each calibrated profile data set with a library of calibrated profile data sets, the plurality of calibrated profile data sets being grouped according to a predetermined biological condition; and

c. determining the progress of the biological condition.

113. A method for establishing a descriptive record for an agent comprising:

a. selecting a population of cells;

b. subjecting the cells to the agent; and

c. determining the record according to any of claims 102 through 106 using a standardized baseline profile data set for the biological condition.

114. A method according to claim 113, wherein the composition is a nutraceutical.

115. A method according to claim 113, wherein the composition is a pharmaceutical.

116. A method according to claim 113, wherein the composition is an infectious agent.

117. A method according to claim 113, wherein the composition is a complex mixture.

118. A method according to claim 113, wherein establishing the biological activity of the composition further includes providing a mechanism of action for the composition.

119. A method according to claim 113, wherein establishing the biological activity of the composition further includes providing a mechanism for metabolism for the composition.

120. A method according to claim 113, wherein the composition further comprises a first compound and a second compound and the biological activity results from any of synergism, interference or neutral interaction between the first and second compound.

121. A method according to claim 113, wherein the compound further comprises a plurality of compounds such that the biological activity results from any of synergism, interference or neutral interaction between the compounds.

122. A method according to claim 113, wherein the biological activity of the compound is a toxic effect on the subject.

123. A method of selecting a therapeutic agent from a class of therapeutic agents for administering to a subject so as to change a biological condition in a subject from a first biological condition to a second biological condition; comprising:

a. subjecting a sample from the subject to each of the class of therapeutic agents;

b. determining a descriptive record for each of the samples according to any of claims 102 through 106;

c. comparing each of the calibrated profile data sets to a library of calibrated profile data sets; wherein the library of calibrated profile data sets are grouped according to a predetermined biological condition; and

d. determining which of the therapeutic agents is capable of changing the first biological condition in the subject to the second biological condition in the subject.

124. A method according to claim 122, wherein the first biological condition is a consequence of the adverse effects of any of an infectious agent, a biological warfare agent or an environmental agent and the second biological condition is a reversal of these adverse effects.

125. A method according to claim 122, wherein the library of descriptive records comprise a medical history for a single subject or single condition.

126. A method according to claim 122, wherein the library of descriptive records comprise medical information about a plurality of subjects or conditions.

127. A method according to claim 122, wherein the library of signature profile data sets consist of signature profile data sets from a plurality of subjects.

128. A method for characterizing the biological effectiveness of a single batch of a composition produced by a manufacturing process, comprising: (a) providing a calibrated data profile set according to the method of claim 3; and labeling the batch of the composition by placing the calibrated data profile set on each container in the batch optionally including the signature calibrated profile data set; (b) comparing the calibrated profiled data set with a standardized calibrated profile data set.

129. A method for accessing biological information on a digital storage medium according to claim 88, comprising: making the information available to a user.

~~130. A method according to claim 129, wherein the method further comprises making the information available to the user on any of a network,~~ World Wide Web, email, internet access site or hard copy.

~~131. A method according to claim 128, wherein the method further~~ comprises accessing the information for loading to a second access site.

132. A method according to claim 131, wherein the process for loading includes downloading the information.

133. A method according to claim 129, wherein access to the information is controlled.

134. A method according to claim 133, wherein the process of control includes the use of a password.

135. A method according to claim 129, wherein the user can annotate the available information, the annotation becoming part of the biological information.

136. A method according to claim 129, wherein the user can add one or more records to the data set, the one or more records becoming part of the biological information.

137. A method for consumer evaluation of a product, wherein the consumer evaluation is dependent on a signature profile comprising: (a) forming a descriptive record according to claim 102; (b) identifying the product using a

descriptive record, wherein the panel of gene loci is a signature panel and (c) comparing the calibrated profiled data set with an average calibrated profile data set to provide an explanation of the product.

~~138. A method according to claim 137, wherein the product is promoted according to the signature profile.~~

139. A computer program product for evaluating a biological condition ~~of a subject or for evaluating a biological condition resulting from the use of an~~ agent, including a computer usable medium having computer readable program code thereon, the computer program code; comprising:

a. a program code for classifying a sample from the subject or the agent for an identifiable record;

b. a program code for deriving a first data set, the first profile data set including a plurality of members, each member being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of the biological condition; the profile data set being stored in the record; and

c. a program code for optionally producing a calibrated profile data set for the panel, for storage in the record, each member of the calibrated profile data set being a function of a corresponding member of the first profile data set and a corresponding member of a baseline profile data set for the panel, the calibrated profile data set providing a measure of the biological condition of the subject.

140. A computer system for evaluating a biological condition of a subject or for evaluating a biological condition resulting from the use of an agent, the computer system, comprising:

a. a classification module for classifying a sample from the subject or the agent in an identifiable record

b. a derivative module for deriving a first data set, the first profile data set including a plurality of members, each member being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of the biological condition; and

c. a production module for producing a calibrated profile data set for the panel, wherein each member of the calibrated profile data set is a function of a corresponding member of the first profile data set and a corresponding member of a baseline profile data set for the panel, the calibrated profile data set providing a measure of the biological condition of the subject.

141. A method for analyzing a patient for a biological condition at a remote site, comprising:

a. providing a kit for measuring a profile data base for evaluating a biological condition, the kit including reagents for quantitative analysis of RNA or protein for a panel of gene loci;

b. accessing a centralized database containing baseline profile data sets corresponding to the panel;

c. determining the calibrated profile data set for the patient; and

d. analyzing the biological condition of the patient with respect to a library of calibrated profile data sets.

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Fig 1

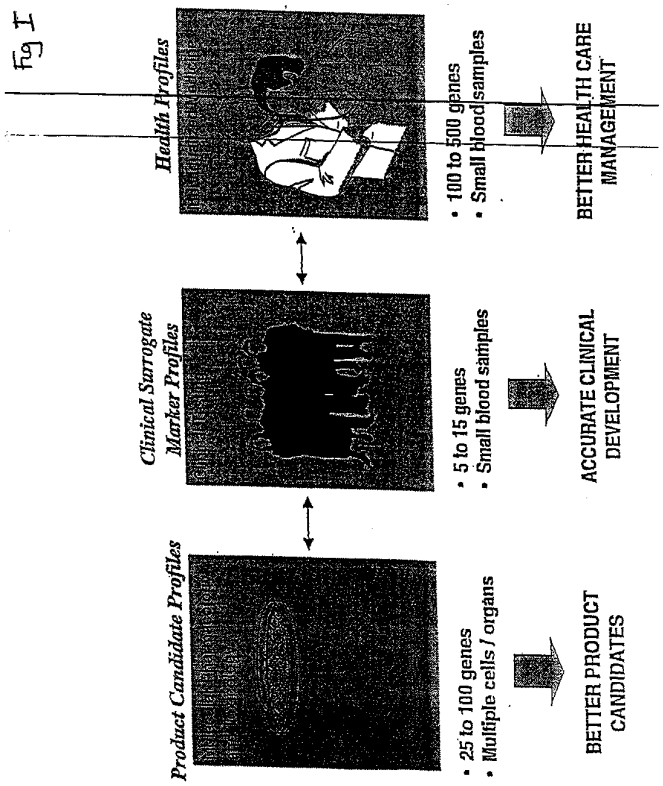
Health Profiles
• 100 to 500 genes
• Small blood samples
BETTER HEALTH CARE MANAGEMENT
Clinical Surrogate Marker Profiles
• 5 to 15 genes
• Small blood samples
ACCURATE CLINICAL DEVELOPMENT
Product Candidate Profiles
• 25 to 100 genes
• Multiple cells / organs
BETTER PRODUCT CANDIDATES

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Fig. 2

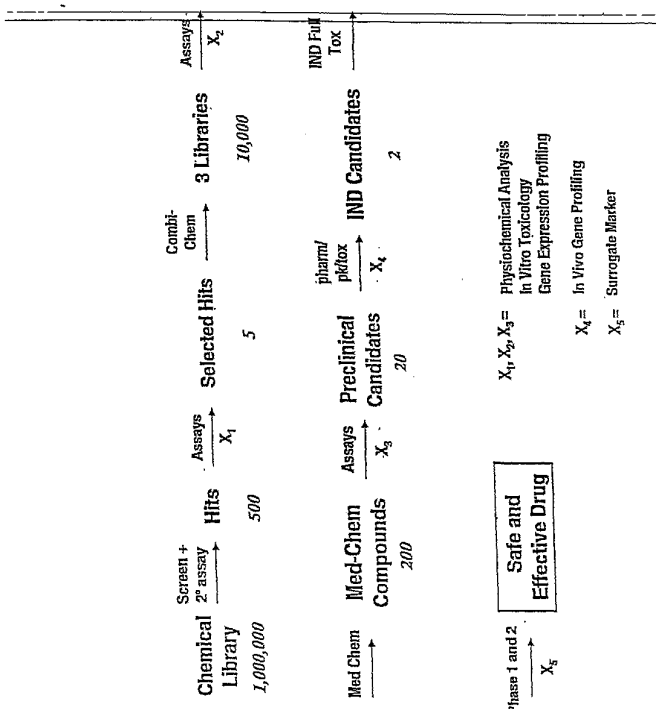

Chemical Library
1,000,000
Screen + 2° assay
Hits
500
Assays
X_1
Selected Hits
5
Combi-Chem
3 Libraries
10,000
Assays
X_2
Leads
2
Med Chem
Med-Chem Compounds
200
Assays
X_3
Preclinical Candidates
20
pharm/ pktox
X_4
IND Candidates
2
IND Full Tox
IND
1
Phase 1 and 2
X_5
Safe and Effective Drug
X_1, X_2, X_3= Physiochemical Analysis
In Vitro Toxicology
Gene Expression Profiling
X_4= In Vivo Gene Profiling
X_5= Surrogate Marker

Fig. 3

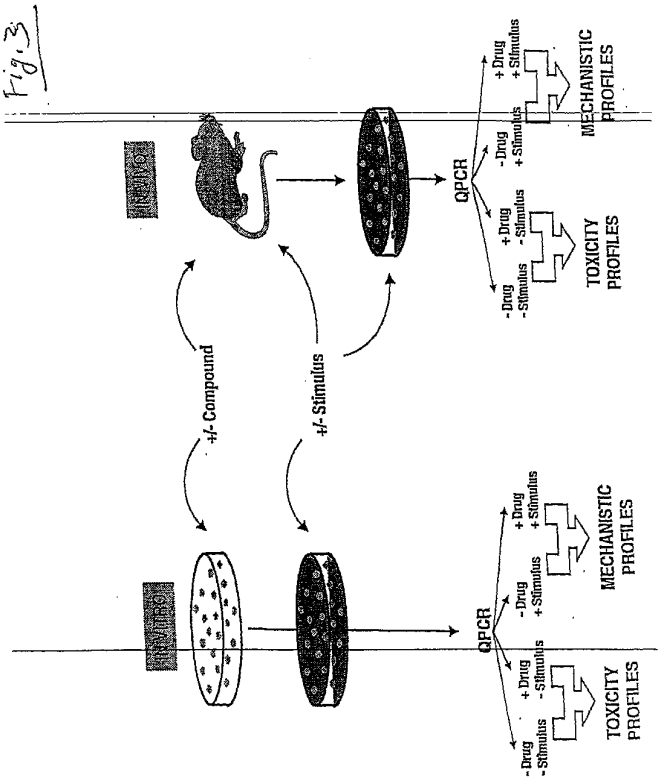
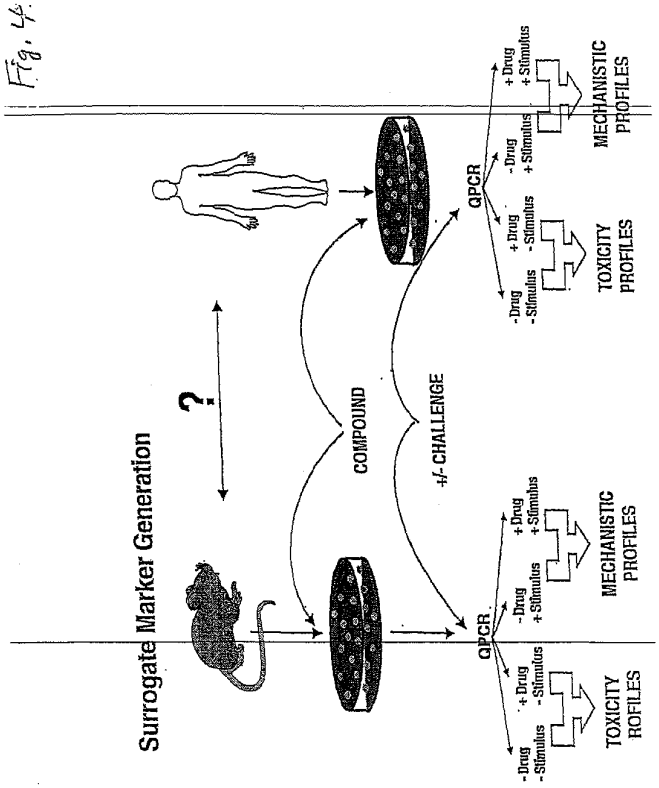
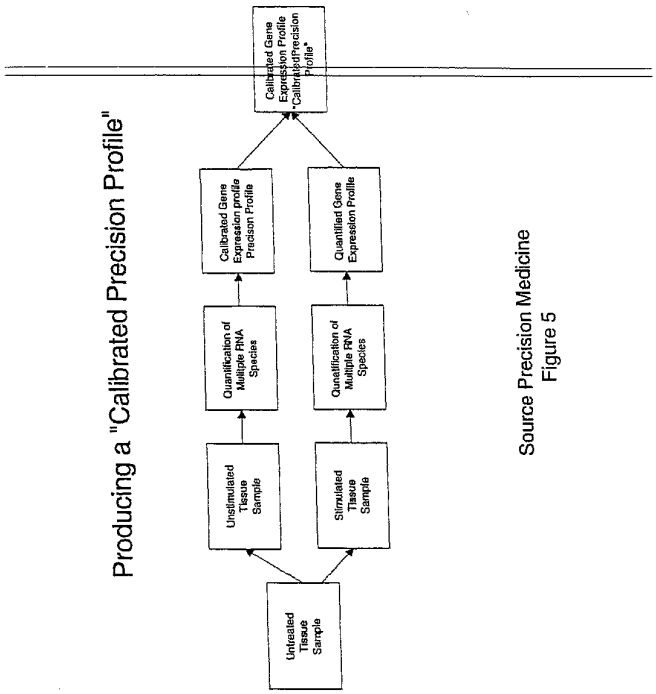

IN VIVO
IN VITRO
+/- Compound
+/- Stimulus
QPCR
+ Drug + Stimulus
- Drug + Stimulus
+ Drug - Stimulus
- Drug - Stimulus
MECHANISTIC PROFILES
TOXICITY PROFILES
QPCR
+ Drug + Stimulus
- Drug + Stimulus
+ Drug - Stimulus
- Drug - Stimulus
MECHANISTIC PROFILES
TOXICITY PROFILES

Fig. 4


Surrogate Marker Generation
?
COMPOUND
+/- CHALLENGE
QPCR
QPCR
+ Drug + Stimulus
- Drug + Stimulus
+ Drug - Stimulus
- Drug - Stimulus
MECHANISTIC PROFILES
TOXICITY PROFILES
MECHANISTIC PROFILES
TOXICITY ROFILES



Source Precision Medicine
Figure 5

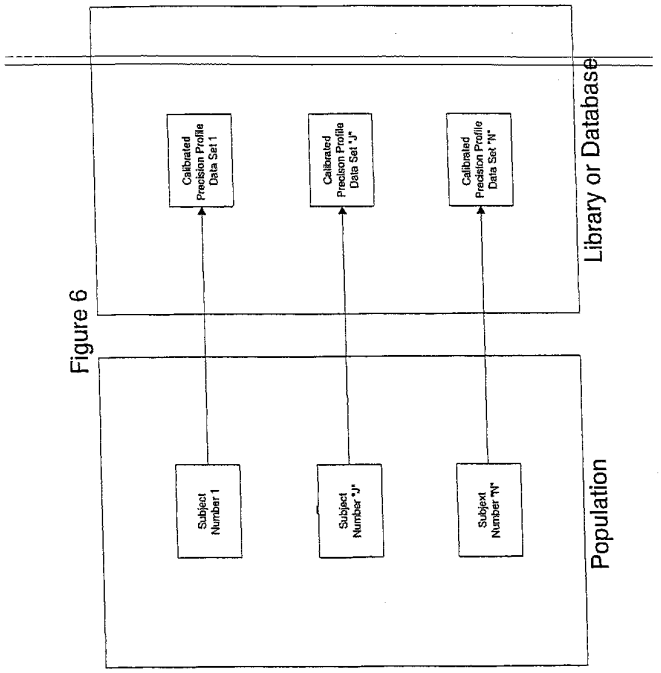

Calibrated Precision Profile Data Set 1
Calibrated Precision Profile Data Set "J"
Calibrated Precision Profile Data Set "N"
Library or Database
Subject Number 1
Subject Number "J"
Subject Number "N"
Population

Figure 6

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Fig 7

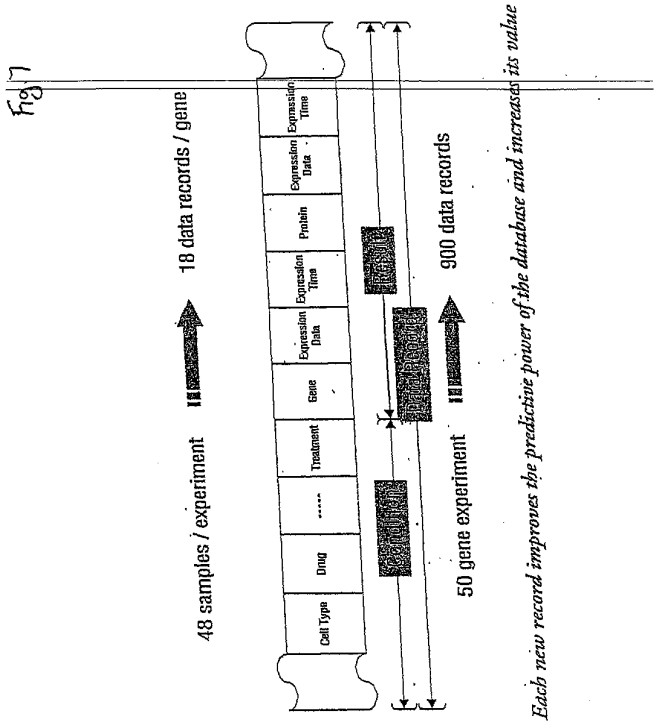
18 data records / gene
48 samples / experiment
Cell Type
Drug
.....
Treatment
Gene
Expression Data
Expression Time
Protein
Expression Data
Expression Time
900 data records
50 gene experiment
Each new record improves the predictive power of the database and increases its value

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Figure 8B

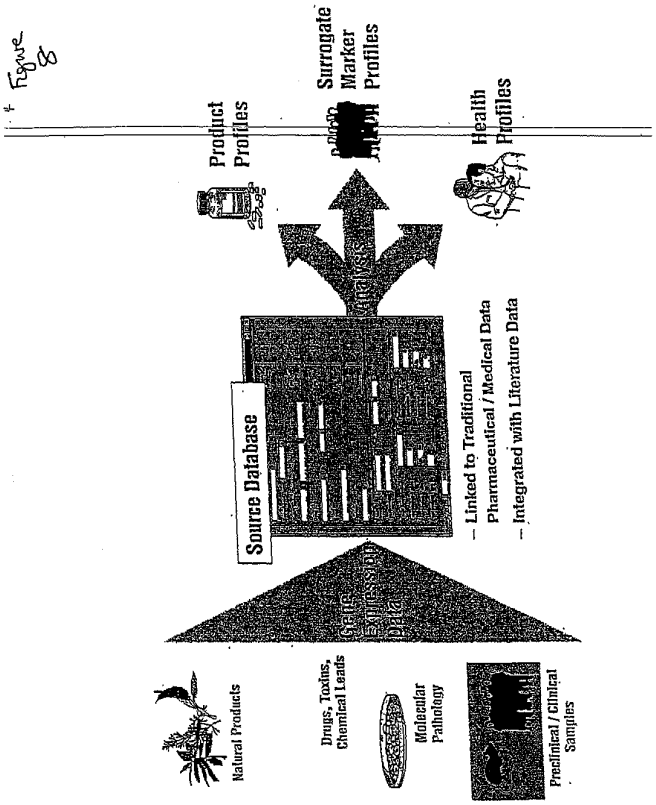

Fig 9

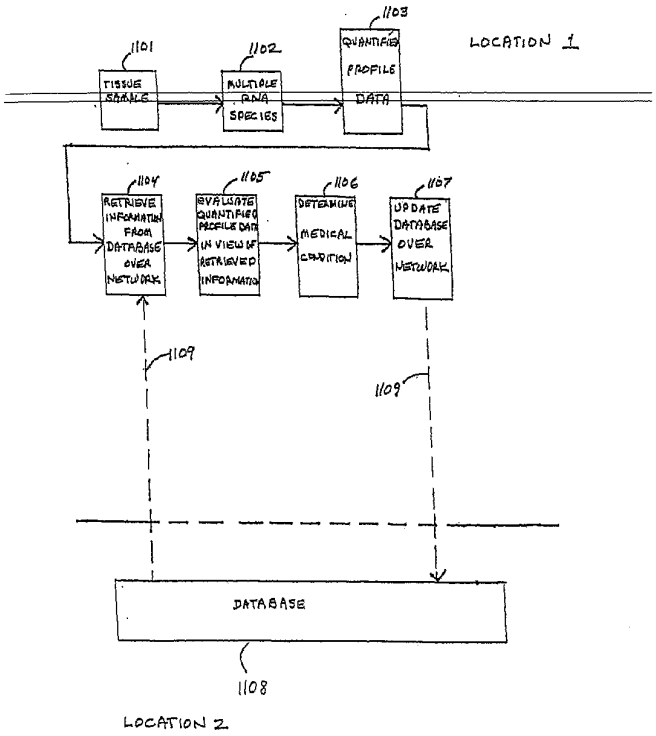
1101
TISSUE SAMPLE
1102
MULTIPLE DNA SPECIES
1103
QUANTIFIED PROFILE DATA
LOCATION 1
1104
RETRIEVE INFORMATION FROM DATABASE OVER NETWORK
1105
EVALUATE QUANTIFIED PROFILE DATA IN VIEW OF RETRIEVED INFORMATION
1106
DETERMINE MEDICAL CONDITION
1107
UPDATE DATABASE OVER NETWORK
1109
1109
DATABASE
1108
LOCATION 2

Fig 10

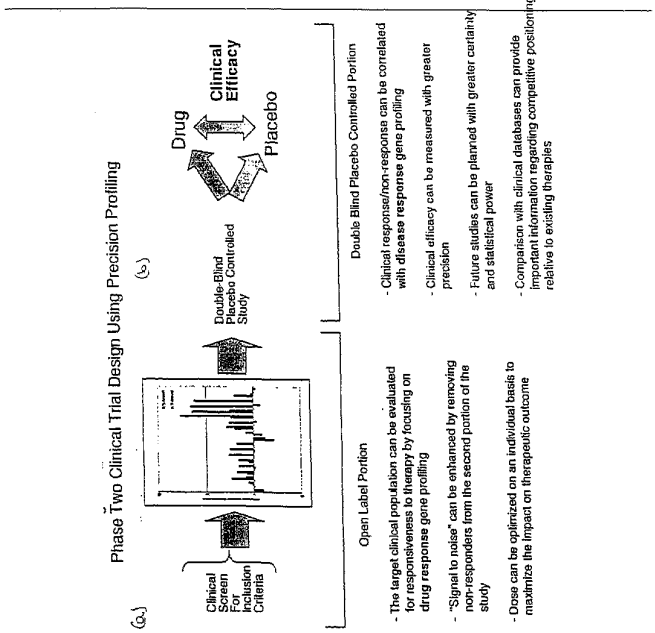
Phase Two Clinical Trial Design Using Precision Profiling
(a)
(b)
Clinical Screen For Inclusion Criteria
Double-Blind Placebo Controlled Study
Drug
Placebo
Clinical Efficacy
Open Label Portion
- The target clinical population can be evaluated for responsiveness to therapy by focusing on drug response gene profiling
- "Signal to noise" can be enhanced by removing non-responders from the second portion of the study
- Dose can be optimized on an individual basis to maximize the impact on therapeutic outcome
Double Blind Placebo Controlled Portion
- Clinical response/non-response can be correlated with disease response gene profiling
- Clinical efficacy can be measured with greater precision
- Future studies can be planned with greater certainty and statistical power
- Comparison with clinical databases can provide important information regarding competitive positioning relative to existing therapies

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Fig. 1(a)

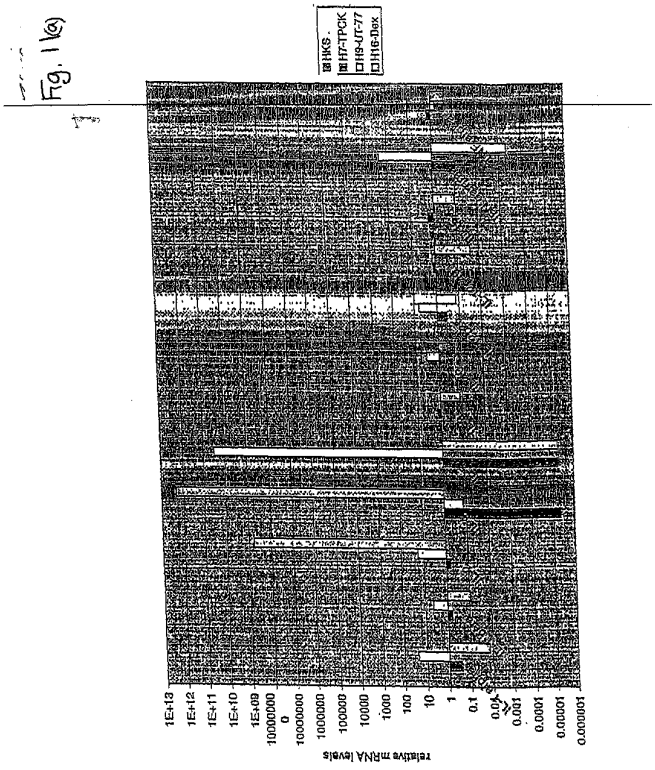
HKS
H7-TPGK
H9-UT-77
H116-Dax
relative mRNA levels
1E+13
1E+12
1E+11
1E+10
1E+09
10000000
0
10000000
1000000
100000
10000
1000
100
10
1
0.1
0.01
0.001
0.0001
0.00001
0.000001

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Fig 11(b)

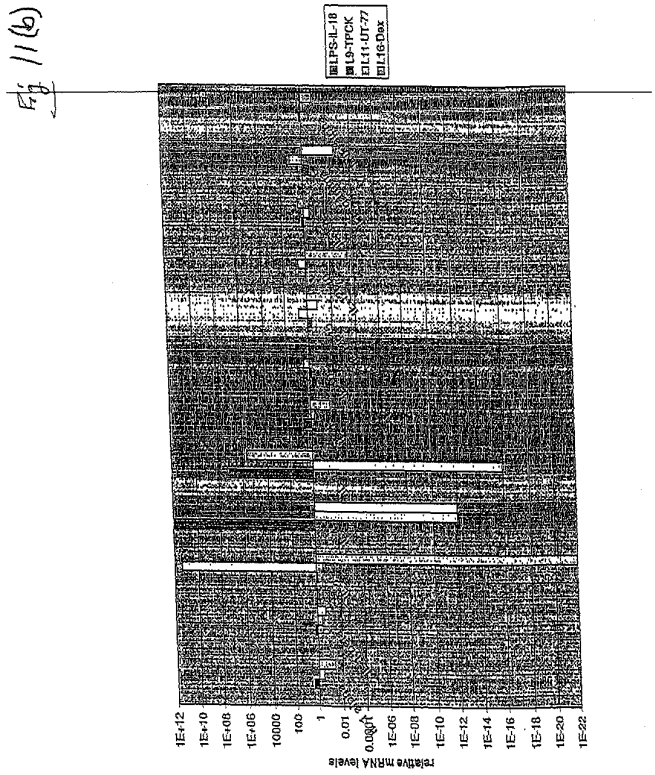
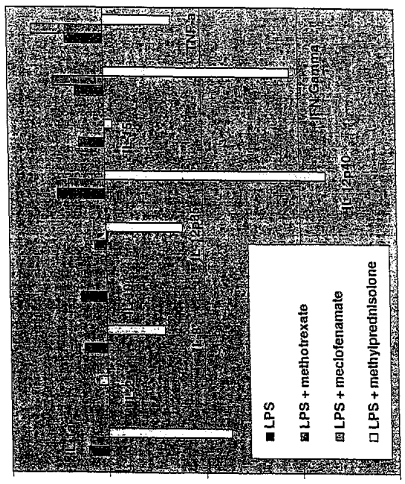

LPS-IL-18
IL9-TPCK
IL11-UT-77
IL16-Dox
1E-12
1E-10
1E-08
1E-06
10000
100
1
0.01
0.0001
1E-05
1E-03
1E-10
1E-12
1E-14
1E-16
1E-18
1E-20
1E-22
relative mRNA levels

Fig 12

Comparative Drug Profiling Shows Differences Among Anti-Inflammatory Drugs with Different Mechanism of Action



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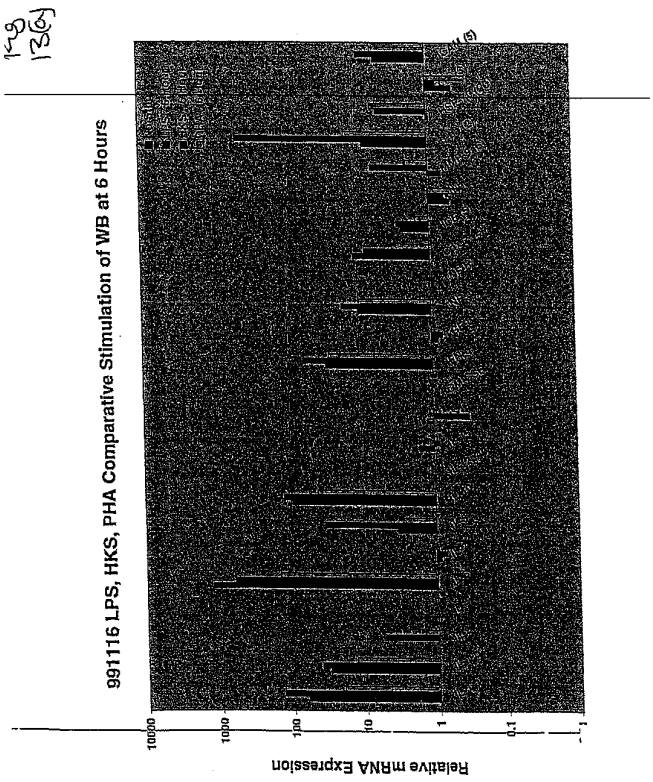
991116 LPS, HKS, PHA Comparative Stimulation of WB at 6 Hours
Relative mRNA Expression
10000
1000
100
10
1
0.1

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13(b)

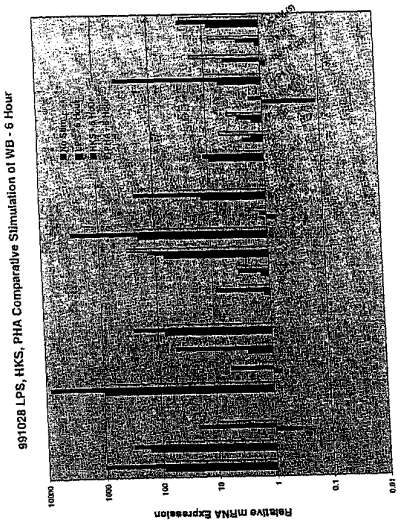
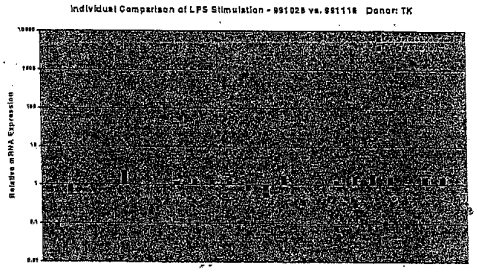


Fig 13b

(13C)


Individual Comparison of LPS Stimulation - 991028 vs. 991118 Donor: TK
Relative mRNA Expression

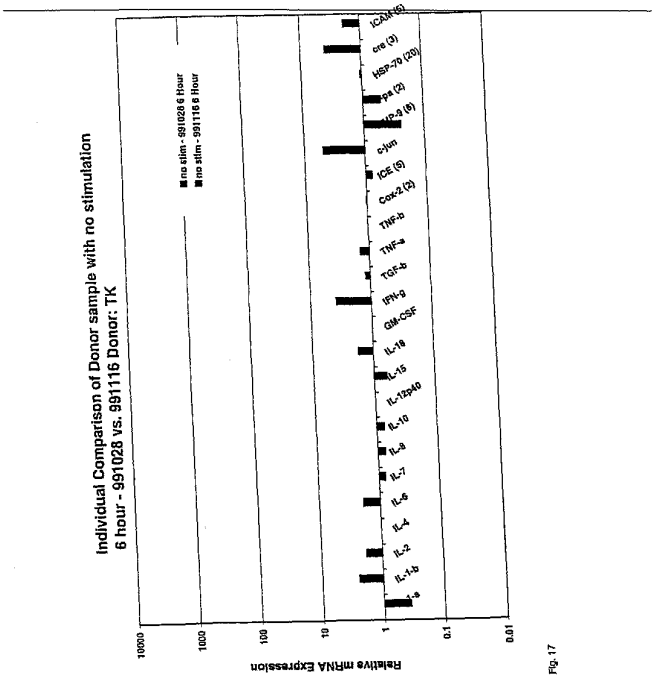

Individual Comparison of Donor sample with no stimulation
6 hour - 991028 vs. 991116 Donor: TK
no stim - 991028 6 Hour
no stim - 991116 6 Hour
ICAM-1(6)
cre (3)
HSP-70 (20)
PS (2)
MIP-3 (9)
c-jun
ICE (5)
Cox-2 (2)
TNF-b
TNF-a
TGF-b
IFN-g
GM-CSF
IL-18
IL-15
IL-12p40
IL-10
IL-8
IL-7
IL-6
IL-4
IL-2
IL-1-b
IL-a
10000
1000
100
10
1
0.1
0.01
Relative mRNA Expression

Fig. 17

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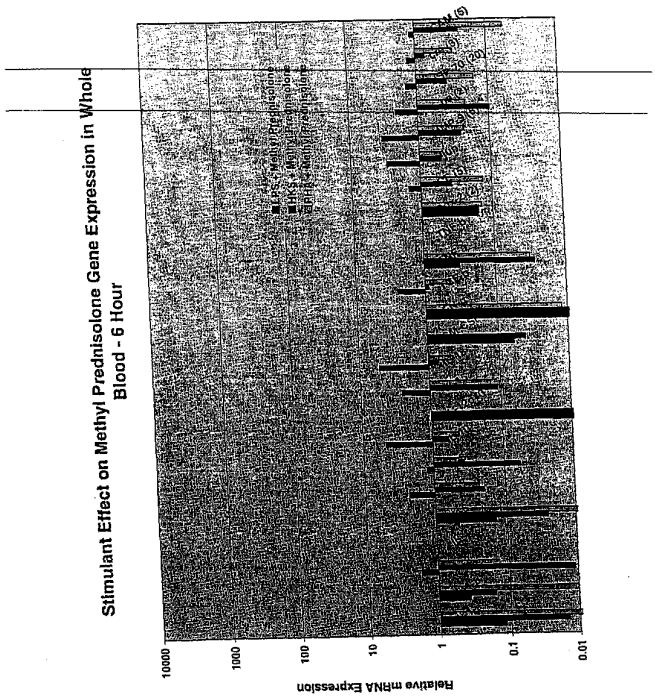
Stimulant Effect on Methyl Prednisolone Gene Expression in Whole Blood - 6 Hour
Relative mRNA Expression
10000
1000
100
10
1
0.1
0.01

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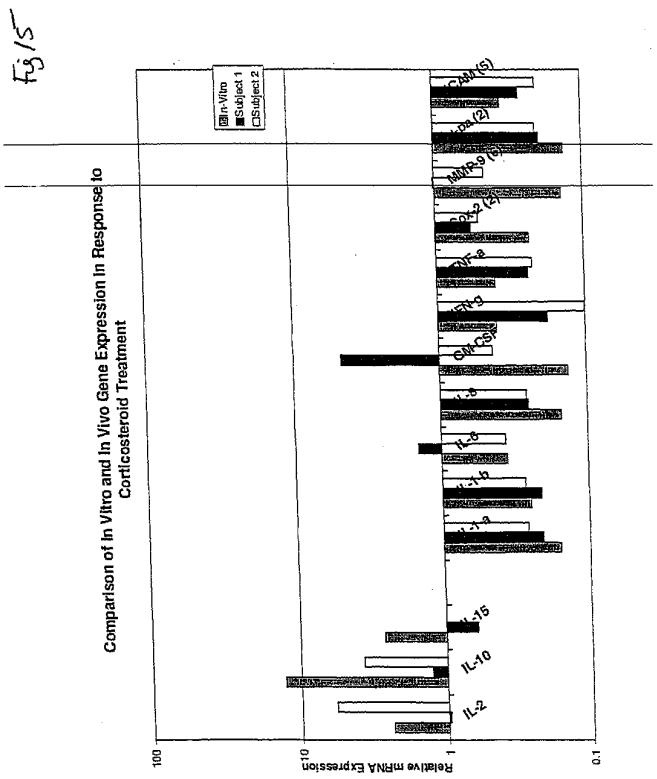
Fig 15


Comparison of In Vitro and In Vivo Gene Expression in Response to Corticosteroid Treatment
In-Vitro
Subject 1
Subject 2
ICAM (S)
MMP-9 (S)
COX-2 (S)
TNF-a
IFN-g
GM-CSF
IL-6
IL-5
IL-15
IL-10
IL-2
Relative mRNA Expression
100
10
1
0.1

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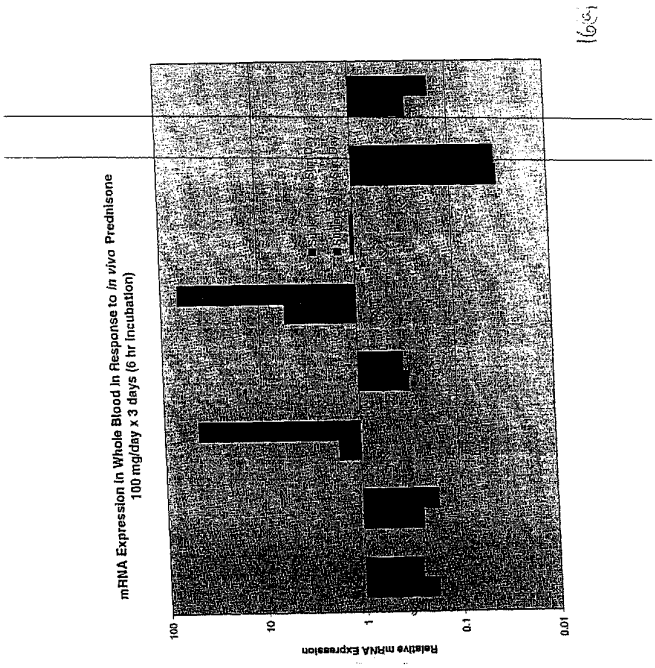
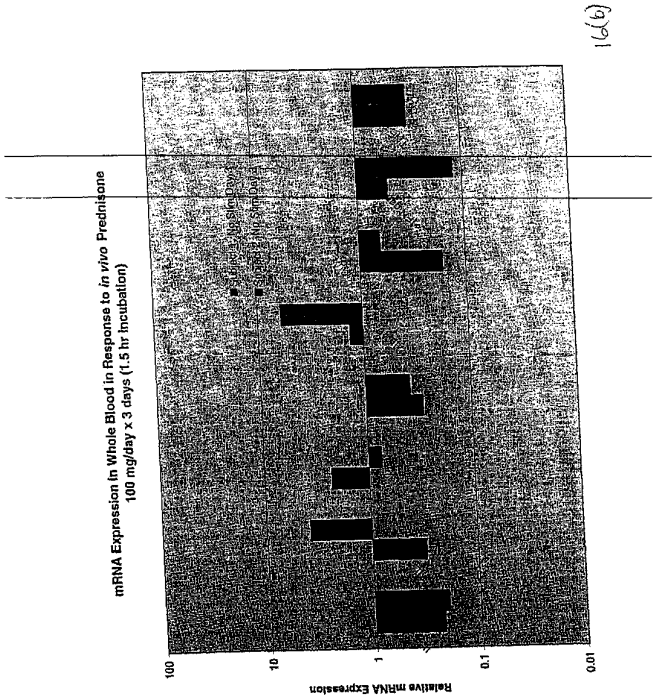
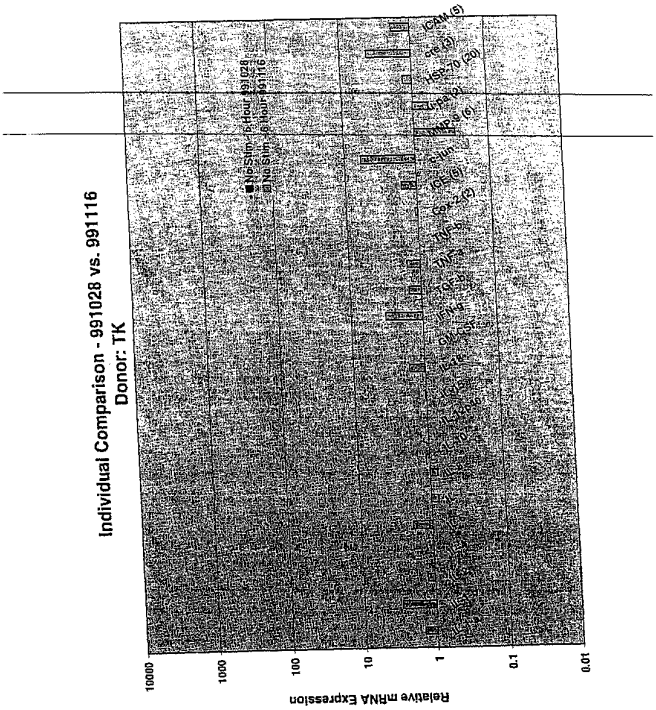

mRNA Expression in Whole Blood in Response to in vivo Prednisone
100 mg/day x 3 days (6 hr incubation)
Relative mRNA Expression
100
10
1
0.1
0.01


mRNA Expression In Whole Blood in Response to in vivo Prednisone
100 mg/day x 3 days (1.5 hr Incubation)
Relative mRNA Expression
100
10
1
0.1
0.01

17


Individual Comparison - 991028 vs. 991116
Donor: TK
991028
991116
Relative mRNA Expression
10000
1000
100
10
1
0.1
0.01

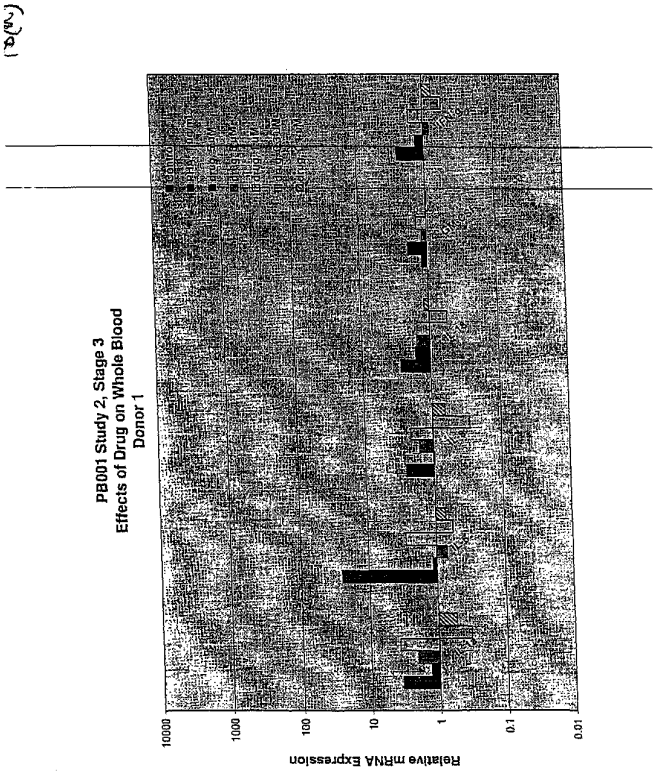

PB001 Study 2, Stage 3
Effects of Drug on Whole Blood
Donor 1
Relative mRNA Expression
10000
1000
100
10
1
0.1
0.01

図6(b)

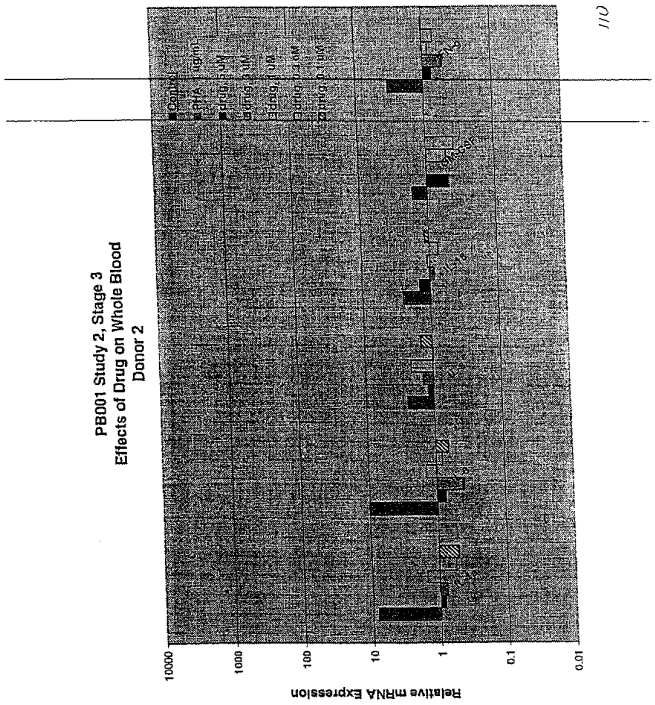
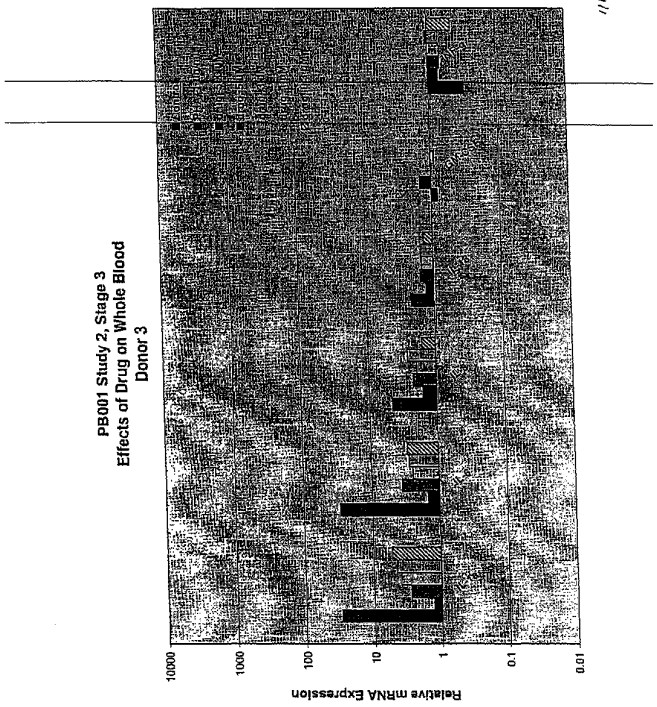
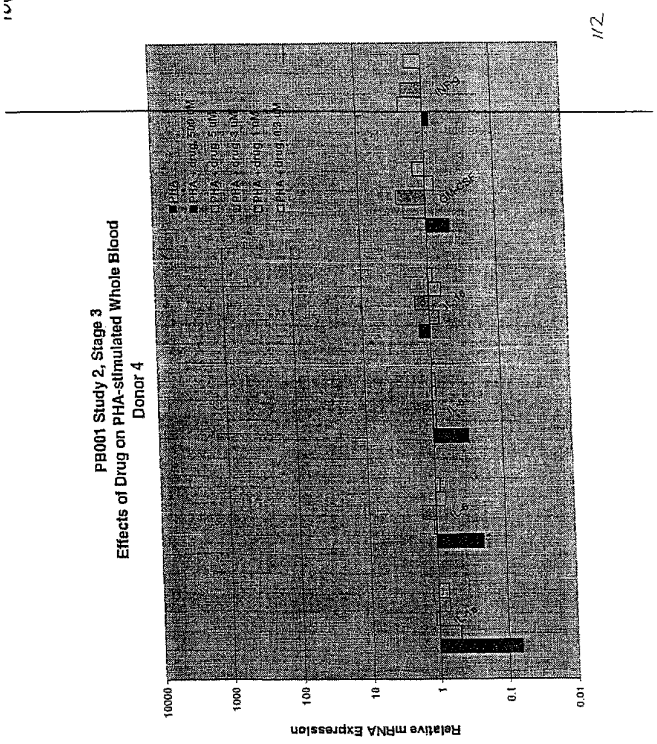
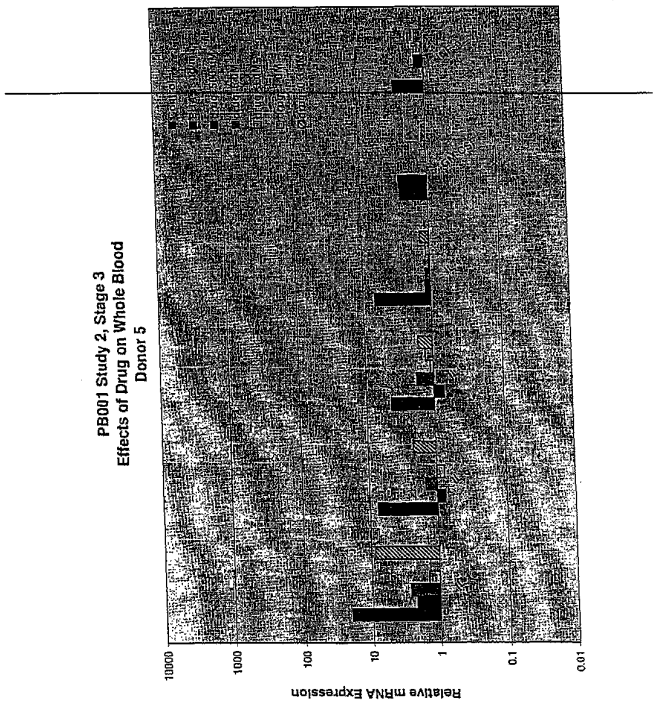

PB001 Study 2, Stage 3
Effects of Drug on Whole Blood
Donor 2
Relative mRNA Expression
10000
1000
100
10
1
0.1
0.01

図15C


PB001 Study 2, Stage 3
Effects of Drug on Whole Blood
Donor 3
Relative mRNA Expression
10000
1000
100
10
1
0.1
0.01


PB001 Study 2, Stage 3
Effects of Drug on PHA-stimulated Whole Blood
Donor 4
Relative mRNA Expression
10000
1000
100
10
1
0.1
0.01

27/36


PB001 Study 2, Stage 3
Effects of Drug on Whole Blood
Donor 5
Relative mRNA Expression
10000
1000
100
10
1
0.1
0.01

19(a)

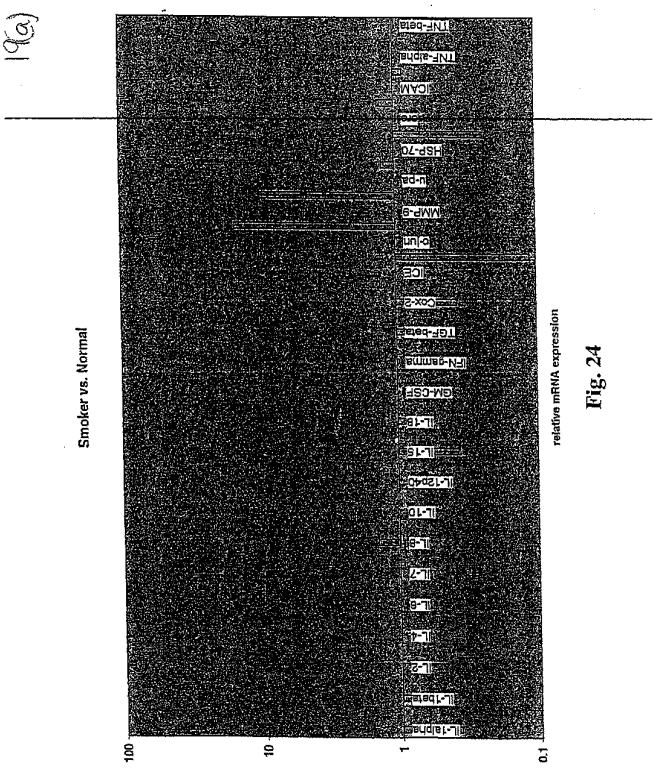

Smoker vs. Normal
IL-1alpha
IL-1beta
IL-2
IL-4
IL-8
IL-7
IL-9
IL-10
IL-12p40
IL-15
IL-18
GM-CSF
IFN-gamma
TGF-beta
Cox-2
ICE
cJun
MMP-9
LPS
HSP-70
Crp
ICAM
TNF-alpha
TNF-beta
100
10
1
0.1
relative mRNA expression

Fig. 24

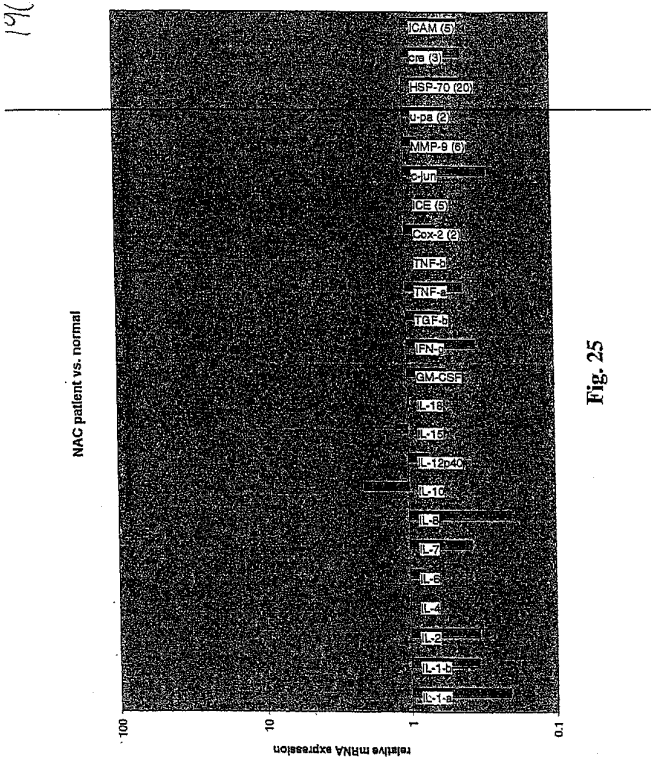

NAC patient vs. normal
ICAM (5)
cre (3)
HSP-70 (20)
u-pa (2)
MMP-9 (6)
c-jun
ICE (5)
Cox-2 (2)
TNF-b
TNF-a
TGF-b
IFN-g
GM-CSF
IL-18
IL-15
IL-12p40
IL-10
IL-8
IL-7
IL-6
IL-4
IL-2
IL-1-b
IL-1-a
100
10
1
0.1
relative mRNA expression

Fig. 25

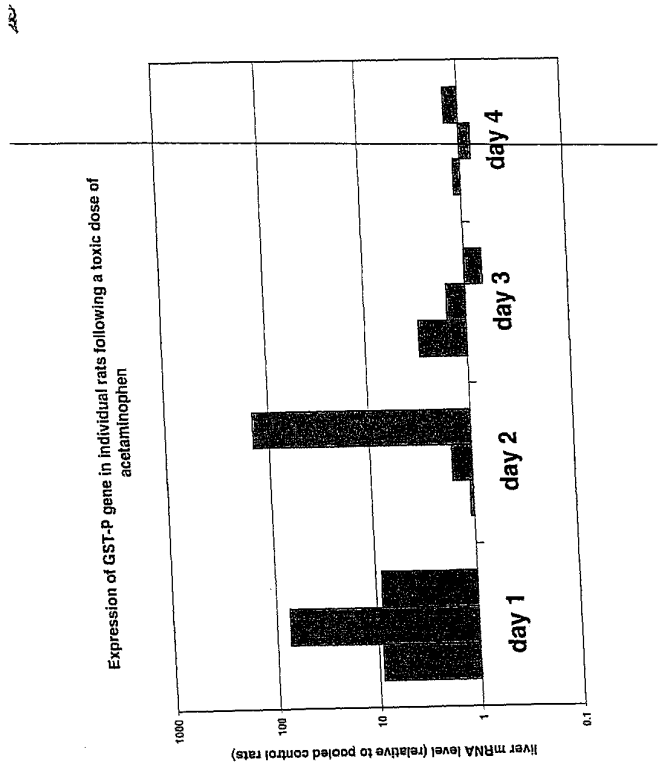

Expression of GST-P gene in individual rats following a toxic dose of acetaminophen
day 4
day 3
day 2
day 1
1000
100
10
1
0.1
liver mRNA level (relative to pooled control rats)

Fig 4

Comparative Herbal Profiling Shows Differences Among Anti-Inflammatory Herbs Such as Echinacea, Arnica and Siberian Ginseng

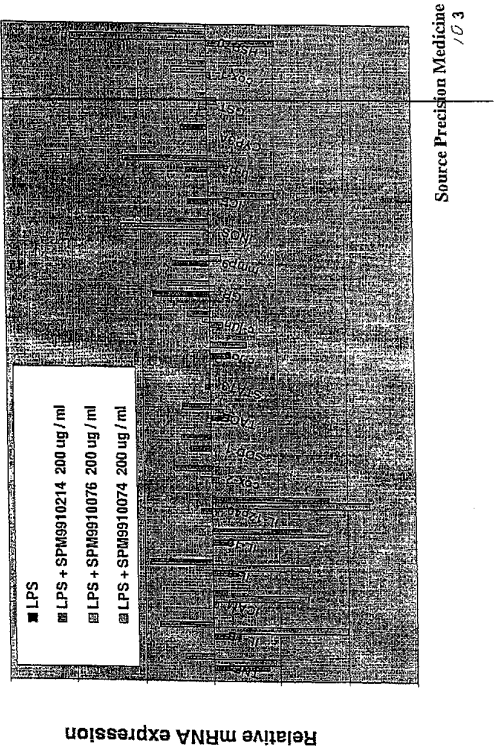
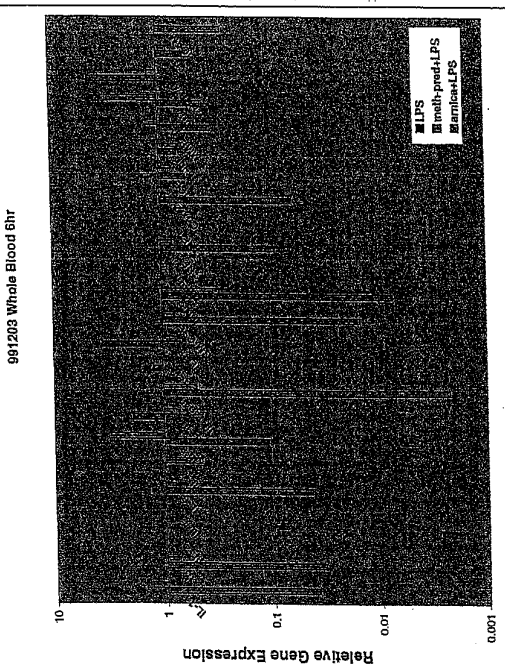
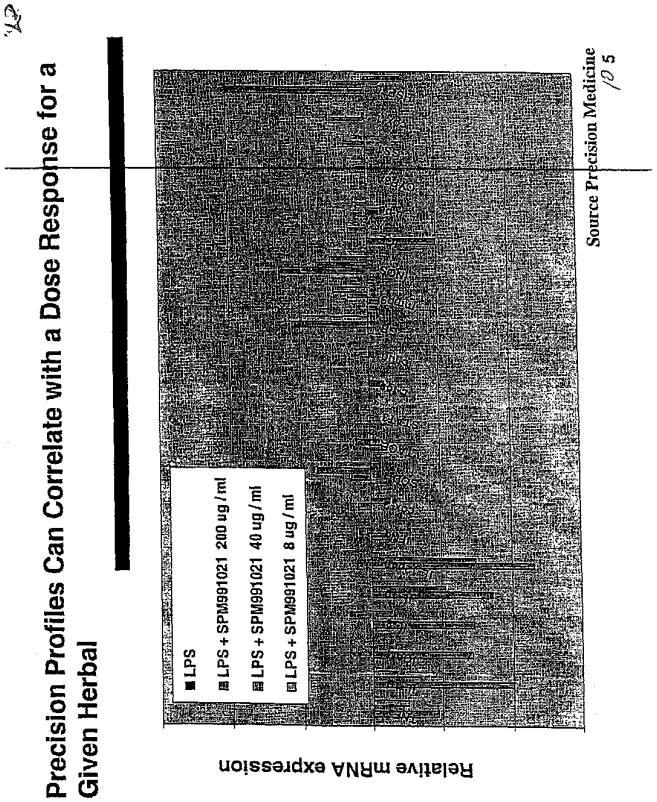


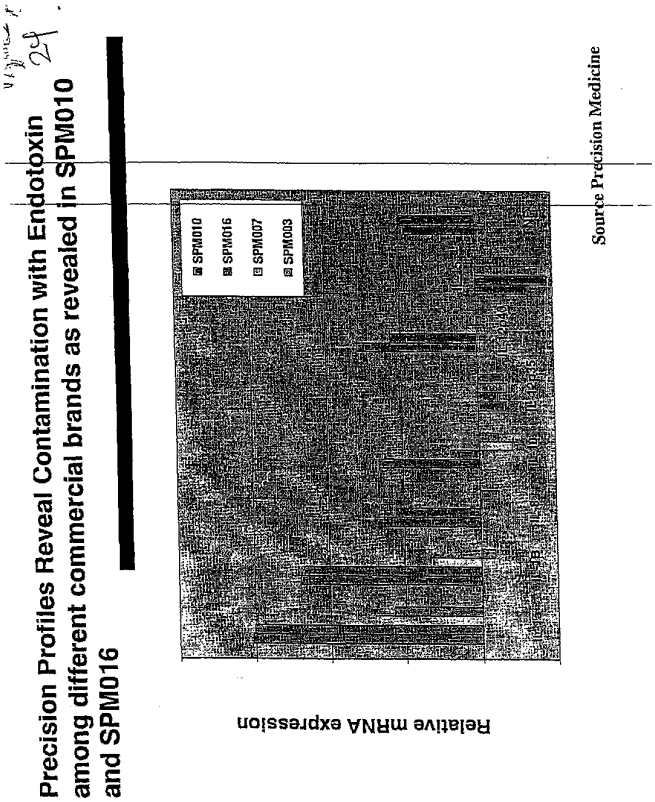
Fig.22


991203 Whole Blood 6hr
LPS
meth-pred+LPS
arnica+LPS
Relative Gene Expression
10
1
0.1
0.01
0.001

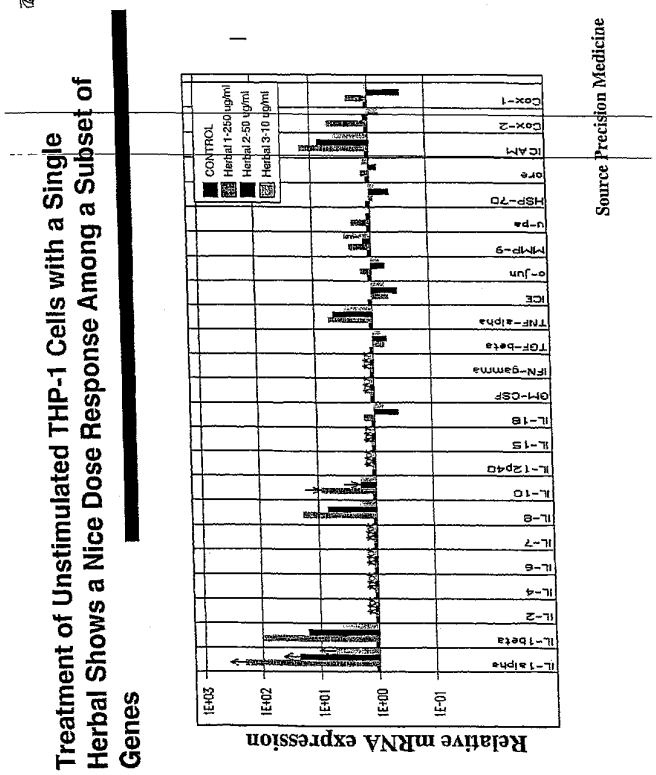
33/136


Precision Profiles Can Correlate with a Dose Response for a Given Herbal
Relative mRNA expression
LPS
LPS + SPM991021 200 ug / ml
LPS + SPM991021 40 ug / ml
LPS + SPM991021 8 ug / ml
Source Precision Medicine

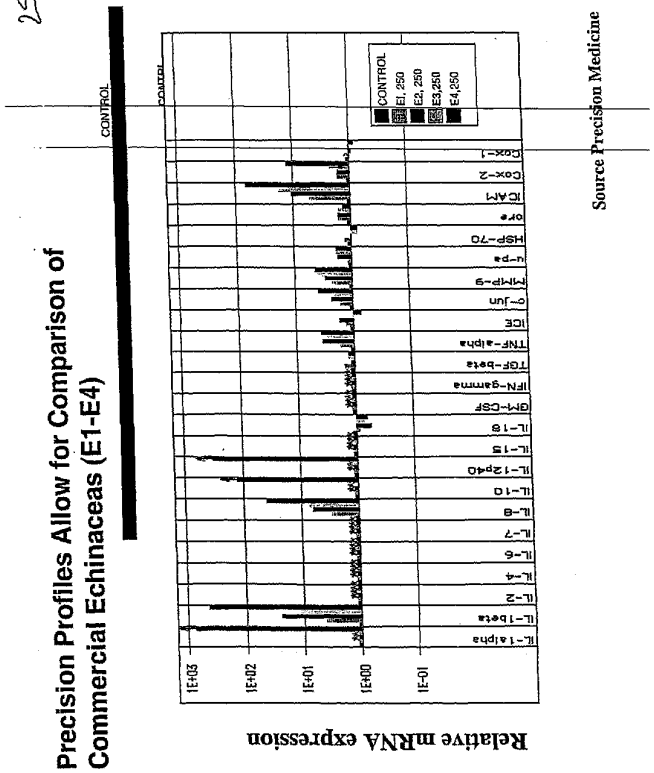
34/36


Precision Profiles Reveal Contamination with Endotoxin among different commercial brands as revealed in SPM010 and SPM016
SPM010
SPM016
SPM027
SPM003
Relative mRNA expression
Source Precision Medicine

35/36


Treatment of Unstimulated THP-1 Cells with a Single Herbal Shows a Nice Dose Response Among a Subset of Genes
Source Precision Medicine
CONTROL
Herbal 1-250 (ug/ml
Herbal 2-50 (g/ml
Herbal 3-10 (g/ml
Relative mRNA expression
1E+03
1E+02
1E+01
1E+00
1E-01
IL-1alpha
IL-1beta
IL-2
IL-4
IL-6
IL-7
IL-8
IL-10
IL-12p40
IL-15
IL-18
GM-CSF
IFN-gamma
TGF-beta
TNF-alpha
ICE
c-Jun
MMP-9
u-Pa
HSP-70
ore
ICAM1
Cox-2
Cox-1

25(C)


Precision Profiles Allow for Comparison of Commercial Echinaceas (E1-E4)
CONTROL
Relative mRNA expression
1E-03
1E-02
1E-01
1E+00
1E-01
CONTROL
E1, 250
E2, 250
E3,250
E4,250
IL-1alpha
IL-1beta
IL-2
IL-4
IL-6
IL-7
IL-8
IL-10
IL-12p40
IL-15
IL-18
GM-CSF
IFN-gamma
TGF-beta
TNF-alpha
ICE
c-Jun
MMP-9
u-pa
HSP-70
ore
ICAM1
Cox-2
Cox-1
Source Precision Medicine

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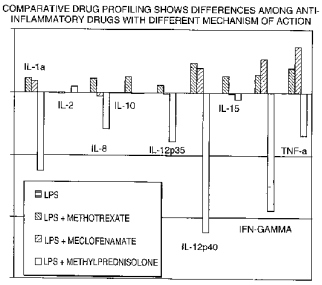
[Continued on next page]

(54) Title: SYSTEMS AND METHODS FOR CHARACTERIZING A BIOLOGICAL CONDITION OR AGENT USING CALIBRATED GENE EXPRESSION PROFILES



(57) Abstract: The invention provides a method, for evaluating a biological condition of a subject, comprising: a. obtaining from the subject a sample having at least one of RNAs and proteins; b. deriving from the sample a first profile data set, the first profile data set including a plurality of members, each member being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of the biological condition; and c. producing a calibrated profile data set for the panel, wherein each member of the calibrated profile data set is a function of a corresponding member of the first profile data set and a corresponding member of a baseline profile data set for the panel, the calibrated profile data set providing a measure of the biological condition of the subject.

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SYSTEMS AND METHODS FOR CHARACTERIZING A BIOLOGICAL CONDITION OR AGENT USING CALIBRATED GENE EXPRESSION PROFILES

Technical Field

A method is provided for identifying reproducible patterns of variation of gene expression that are informative by virtue of the degree of variation observed in a calibrated data set. The variations may be correlated with other non- genetic indications such as clinical indicators (for humans) of a traditional nature but are not required per se to be causative.

Background Art

There has been substantial discussion including congressional hearings concerning medical errors. One source of medical errors include errors with medications. Upwards of 98,000 hospitalized patients annually have been documented to be victims of medication errors (Statement of the American Pharmaceutical Association to the Senate Appropriations Committee Labor, health and Human Services Education Subcommittee Hearing on Medical Errors December 13, 1999). These errors include problems arising from drug interactions for a particular patient taking more than one drug, problems concerning the response of an individual to a particular drug and incorrect medication for a particular condition. Medical errors further arise as a result of misdiagnosis. This may occur as a result of insensitive diagnostic techniques or a wide range of interpersonal variability in the manner in which a clinical state is manifest. At present, there are few tools available for optimizing prognosis, diagnosis and treatment of a medical condition taking into account the particular phenotype and genotype of an individual.

There has been increasing interest in herbal drugs or nutraceuticals. These are often grown in developing countries and undergo little or no quality control. It is frequently the case that one batch of a nutraceutical may be effective, there is no assurance that a second batch will be effective Moreover, analysis of nutraceuticals is problematic because these drugs are complex mixtures in which little is known with respect to the active agent.

All new therapeutic agents require some form of clinical trials. It is known that a drug for treating tumor that is tested in a clinical trial using standard recruiting techniques for patients, may in fact show only limited efficacy. If the beneficial effect observed in a clinical population is too small, the drug will not receive approval by the Food and Drug Administration for use in the population at large. However, the small beneficial effect observed may in fact be an artifact of the clinical trial design or the clinical endpoint in the population of patients. It would be desirable to have criteria for screening patients as they enter a clinical trial to ensure that the beneficial effect of a drug if it exists may be detected and quantified.

Summary of the Invention

In a first embodiment of the invention there is provided a method, for evaluating a biological condition of a subject, that includes: obtaining from the subject a sample having at least one of RNAs and proteins; deriving from the sample a first profile data set, the first profile data set including a plurality of members, each member being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of the biological condition; and producing a calibrated profile data set for the panel, wherein each member of the calibrated profile data set is a function of a corresponding member of the first profile data set and a corresponding member of a baseline profile data set for the panel, the calibrated profile data set providing a measure of the biological condition of the subject.

In a preferred embodiment, a method is provided for evaluating a biological condition of a subject, that includes obtaining from the subject a first sample having at least one of fluid, cells and active agents; applying the first sample or a portion thereof to a defined population of indicator cells; obtaining from the indicator cells a second sample containing at least one of RNAs or proteins; deriving from the second sample a first profile data set, the first profile data set including a plurality of members, each member being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables

measurement of the biological condition; and producing a calibrated profile data set for the panel, wherein each member of the calibrated profile data set is a function of a corresponding member of the first profile data set and a corresponding member of a baseline profile data set for the panel, the calibrated profile data set providing a measure of the biological condition of the subject.

In a preferred embodiment, a method is provided for evaluating a biological condition affected by an agent, the method including: obtaining, from a target population of cells to which the agent has been administered, a sample having at least one of RNAs and proteins; deriving from the sample a first profile data set, the first profile data set including a plurality of members, each member being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of the biological condition; and producing a calibrated profile data set for the panel, wherein each member of the calibrated profile data set is a function of a corresponding member of the first profile data set and a corresponding member of a baseline profile data set for the panel, the calibrated profile data set providing a measure of the biological condition as affected by the agent.

In a preferred embodiment, a method is provided for evaluating the effect on a biological condition by a first agent in relation to the effect by a second agent, including: obtaining, from first and second target populations of cells to which the first and second agents have been respectively administered, first and second samples respectively, each sample having at least one of RNAs and proteins; deriving from the first sample a first profile data set and from the second sample a second profile data set, the profile data sets each including a plurality of members, each member being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of the biological condition; and producing for the panel a first calibrated profile data set and a second profile data set, wherein (i) each member of the first calibrated profile data set is a function of a corresponding member of the first profile data set and a corresponding member of a first baseline profile data set for the panel, and (ii)

each member of the second calibrated profile data set is a function of a corresponding member of the second profile data set and a corresponding member of a second baseline profile data set for the panel, the calibrated profile data sets providing a measure of the effect by the first agent on the biological condition in relation to the effect by the second agent.

In a preferred embodiment, a method of conducting a clinical trial of an agent, is provided, including: causing the blind administration of a selected one of a placebo and the agent to each candidate of a pool of subjects; and using quantitative gene expression to monitor an effect of such administration.

In a preferred embodiment, a digital storage medium is provided on which is stored a computer readable calibrated profile data set, wherein: the calibrated profile data set relates to a sample having at least one of RNAs and proteins derived from a target cell population to which an agent has been administered; the calibrated profile data set includes a first plurality of members, each member being a quantitative measure of a change in an amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of a biological condition as affected by administration of the agent.

In a preferred embodiment, a digital storage medium is provided on which is stored a plurality of records R_i relating to a population of subjects, each record R_i corresponding to a distinct instance P_i of a computer readable profile data set P wherein: each instance P_i of the profile data set P relates to a distinct sample derived from a subject, the sample having at least one of RNAs and proteins; the profile data P set includes a plurality of members M_j, each member M_j being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of a biological condition; each record R_i includes, for each member M_{ij} of a corresponding distinct instance P_i of the profile data set P, a value corresponding to the value of the member M_{ij}; and each record R_i also includes a reference to a characteristic of the subject relative to the record, the characteristic being at least one of age group, gender, ethnicity, geographic location, diet, medical disorder, clinical indicator,

medication, physical activity, body mass, and environmental exposure.

In a preferred embodiment, a digital storage medium is provided on which is stored a large number of computer readable profile data sets, wherein each profile data set relates to a sample derived from a target cell population to which has been administered an agent, the sample having at least one of RNAs and proteins; each profile data set includes a plurality of members, each member being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of a biological condition; and the panel is the same for all profile data sets.

In a preferred embodiment of the invention, a method is provided for evaluating a biological condition of a subject, based on a sample from the subject, the sample having at least one of RNAs and proteins, the method including: deriving from the sample a first instance of a profile data set, the profile data set including a plurality of members, each member being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of the biological condition; and producing a first instance of a calibrated profile data set for the panel, wherein each member of an instance of the calibrated profile data set is a function of a corresponding member of an instance of the profile data set and a corresponding member of an instance of a baseline profile data set for the panel, the calibrated profile data set providing a measure of the biological condition of the subject; accessing data in a condition database, the condition database having a plurality of records relating to a population of subjects, each record corresponding to a distinct instance of the calibrated profile data set; and evaluating the first instance of the calibrated profile data set in relation to data in the condition database.

In a preferred embodiment of the invention, a method is provided of displaying quantitative gene expression analysis data associated with measurement of a biological condition, the method including: identifying a first profile data set pertinent to the gene expression analysis data, the first profile data set including a plurality of members, each member being a quantitative

measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of the biological condition; producing a calibrated profile data set for the panel, wherein each member of the calibrated profile data set is a function of a corresponding member of the first profile data set and a corresponding member of a baseline profile data set for the panel, the calibrated profile data set providing a measure of the biological condition of the subject; and displaying the calibrated profile data set in a graphical format.

A preferred embodiment is directed to a descriptive record of a change in a biological condition, that includes: a first set of numerical gene expression values for a panel of gene loci, each value in the set corresponding to a single gene locus in a panel of gene loci, the set of values forming a profile data set for a population of cells subjected to a first biological condition; a second set of numerical gene expression values for the panel of gene loci, each value in the set corresponding to a single gene locus, the set of values forming a baseline profile data set for a second population of cells subjected to a second biological condition, the second set of values optionally being an average for multiple gene expression values from multiple populations of cells for each locus in the panel; and a third set of numbers corresponding to the ratio of the first set of values and the second set of values with respect to each gene locus in the panel, the third set being a calibrated profile data set; the profile data set and the calibrated profile data set being descriptive of the first biological condition with respect to the second biological condition.

In a preferred embodiment, a method for diagnosing a biological condition of a subject is provided that includes: obtaining a sample from a subject; subjecting a population of cells to the sample and determining the presence of a first biological condition with respect to a second biological condition according to any of the above claims.

In a preferred embodiment, a method is provided for diagnosing a susceptibility for a biological condition in a subject, that includes obtaining a sample from the subject; creating a descriptive record, according to the above, wherein the baseline set of values is an average of second values contained in a

library of descriptive records for the second biological condition; the library containing a plurality of descriptive records grouped according to a predetermined biological condition; comparing the calibrated profile data set of the subject with the library of calibrated profile data sets and diagnosing the susceptibility of the subject.

In a preferred embodiment, a method is provided for monitoring the progress of a biological condition, including: creating a plurality of descriptive records, according to the above; wherein each set of first values is determined at preselected time intervals with respect to the first record; comparing each calibrated profile data set with a library of calibrated profile data sets, the plurality of calibrated profile data sets being grouped according to a predetermined biological condition; and determining the progress of the biological condition with respect to gene expression.

In a preferred embodiment, a method is provided for establishing the biological activity of a composition, including: selecting a population of cells; subjecting the cells to the composition; and determining the record according to the above description using a standardized baseline profile data set for the biological condition.

In a preferred embodiment, a method is provided for determining which therapeutic agent from a choice of a plurality of therapeutic agents to administer to a subject so as to change a biological condition in a subject from a first biological condition to a second biological condition; including: subjecting a sample from the subject to each of a plurality of therapeutic agents; determining a descriptive record for each of the samples according to any of the above described methods, comparing each of the calibrated profile data sets to a library of calibrated profile data sets, the library of calibrated data sets being grouped according to a predetermined biological condition; and determining which of the therapeutic agents is capable of changing the first biological condition in the subject to the second biological condition in the subject.

In a preferred embodiment, a method is provided for characterizing the biological effectiveness of a single batch of a composition produced by a manufacturing process, comprising: providing a fingerprint or signature profile

according to any of the above methods; and labeling the batch of the composition by placing the fingerprint (signature profile) on each container in the batch.

In a preferred embodiment, a method is provided for accessing biological information on a digital storage medium as described above, including: making the information available to a user.

In a preferred embodiment, a method is provided for consumer evaluation of a product, wherein the consumer evaluation is dependent on a signature profile, including: identifying the product using the signature profile.

In a preferred embodiment, a computer program product is provided for evaluating a biological condition of a subject or for evaluating a biological condition resulting from the use of an agent, including a computer usable medium having computer readable program code thereon, the computer program code; including: a program code for classifying a sample from the subject or the agent for an identifiable record; a program code for deriving a first data set, the first profile data set including a plurality of members, each member being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of the biological condition; the profile data set being stored in the record; and a program code for optionally producing a calibrated profile data set for the panel, for storage in the record, each member of the calibrated profile data set being a function of a corresponding member of the first profile data set and a corresponding member of a baseline profile data set for the panel, the calibrated profile data set providing a measure of the biological condition of the subject.

In a preferred embodiment of the invention, a computer system for evaluating a biological condition of a subject or for evaluating a biological condition resulting from the use of an agent is provided, the computer system, including: a classification module for classifying a sample from the subject or the agent in an identifiable record; a derivative module for deriving a first data set, the first profile data set including a plurality of members, each member being a quantitative measure of the amount of a distinct RNA or protein

constituent in a panel of constituents selected so that measurement of the constituents enables measurement of the biological condition; and a production module for producing a calibrated profile data set for the panel, wherein each member of the calibrated profile data set is a function of a corresponding member of the first profile data set and a corresponding member of a baseline profile data set for the panel, the calibrated profile data set providing a measure of the biological condition of the subject.

In a preferred embodiment, a method is provided for analyzing a patient for a biological condition at a remote site, including: providing a kit for measuring a profile data base for evaluating a biological condition, the kit including reagents for quantitative analysis of RNA or protein for a panel of gene loci; accessing a centralized database containing baseline profile data sets corresponding to the panel; determining the calibrated profile data set for the patient; and analyzing the biological condition of the patient.

Preferred embodiments of the invention include the use of calibrated profile databases for determining the biological condition at one site in a subject from a sample taken from a second remote site. The biological condition may include disease, therapeutic interventions, aging, health conditioning and exercise, exposure to toxins, status of infection and health status. For example, calibrated precision profiles may be used to measure a biological condition(s) in one site (for example, the liver) by sampling cells from the same subject, but at a different site not generally considered a target for the biological condition, for example, peripheral blood cells in the case of liver disease.

Preferred embodiments of the invention include the use of calibrated profile databases for determining the biological condition of the subject that includes placing a cell or fluid sample on indicator cells to assess the biological condition, the biological condition including disease, therapeutic interventions, aging, health conditioning and exercise, exposure to toxins, status of infection and health status.

Preferred embodiments of the invention include the use of calibrated profile data bases and profiles to assess, compare and contrast the bioactivities of therapeutic agents and therapeutic agent candidates including comparison of

two agents having unknown properties ; comparison of agents that are complex mixtures against those that are simple mixtures and comparisons of a single agent against a class of agents.

Preferred embodiments of the invention include the use of calibrated profile databases derived from *in vitro* dosing of an agent in indicator cells, or fluids or cells *ex vivo* to predict *in vivo* activities, activities including efficacy and toxicity and further permitting data on short term *in vivo* dosing of agent to predict long-term activities as described herein.

A preferred embodiment of the invention is at least one databases and its uses, the databases containing at least one of calibrated profile data sets and baseline profile data sets for discrete populations identified according to factors including diseases, geography, ethnicity, age and state of health.

A preferred embodiment of the invention is a database corresponding to an individual over time, the uses including managing a personalized health care program.

Additional embodiments include methods of running a clinical trial using calibrated profile data and databases containing calibrated profile data from *in vitro* and *in vivo* studies of the effect of the agent on populations of cells and methods of building a clinical research network that uses calibrated profile data and traditional medical data.

Brief Description of the Drawings

The foregoing features of the invention will be more readily understood by reference to the following detailed description, taken with reference to the accompanying drawings, in which:

Figure 1 is a diagram showing the flow of information from data acquired in molecular pharmacology and toxicology, clinical testing, and use of the data for the application to individualized medicine.

Figure 2 is a diagram showing the drug discovery pathway of new compounds from early leads to likely drug candidates. Although calibrated profile data sets are indicated at the pre-clinical step, gene expression data can be acquired and is useful at any of the stages shown. IND refers to investigative new drug and refers to an early stage in regulatory review.

Figure 3 is a diagram presenting a comparison of *in vivo* and *in vitro* protocols for forming calibrated profile data sets for rapidly assessing product candidate toxicity and efficacy in accordance with several embodiments of the present invention.

Figure 4 is a diagram showing the application of gene expression profiling as a guide to pre-clinical and clinical studies in accordance with an embodiment of the present invention.

Figure 5 is a diagram showing a method in accordance with an embodiment of the present invention for obtaining profile data in the absence of a stimulus and in the presence of a stimulus.

Figure 6 is a diagram showing the creation of a library of profile data associated with a plurality of subjects in accordance with an embodiment of the present invention.

Figure 7 is a diagram illustrating the structure of a profile data record in accordance with an embodiment of the present invention.

Figure 8 is a diagram illustrating a data entry screen for a data record of the type shown in Figure 7 and typical contexts in which data records may be compiled in accordance with embodiments of the present invention.

Figure 9 shows an embodiment of the present invention in which profile data, in either the raw or calibrated form, is evaluated using data from a database that is remotely accessed over a network.

Figure 10 shows a schematic of a phase two clinical trial that utilizes gene expression profiling (a) The right hand panel (b) indicates that the same information may be used in Phase IV or post marketing studies to compare the efficacy of already approved and marketed drugs or to guide the marketing of such therapies; to guide the choice of therapy for an individual subject or population from within a class of appropriate compounds.

Figure 11 is a bar graph that shows a graphical representation in the form of a histogram representing calibrated profile data sets based on quantitative expression of RNA in cells of a whole blood sample using a panel of 12 constituents where each constituent corresponds to a unique gene locus. (a) The blood sample is stimulated *ex vivo* with heat killed staphylococci are further

exposed H7-TPCK, H9-UT-77, or H16-Dex as indicated. The baseline profile data set is a blood sample stimulated *ex vivo* (*in vitro*) with heat killed staphylococci (b) The blood sample is stimulated *ex vivo* with lipopolysaccharide (LPS) and is then further exposed to compounds H7-TPCK, H9-UT-77, or H16-Dex as indicated.

Figure 12 is a bar graph with a logarithmic axis that shows a graphical representation of calibrated profile data sets for whole blood stimulated *ex vivo* with lipopolysaccharide (LPS), using a panel of 9 constituents, each constituent corresponding to a gene locus encoding the gene products indicated, the blood being further exposed to anti-inflammatory agents: methotrexate, meclofenamate and methylprednisolone. The baseline profile data set is derived from LPS stimulated (but otherwise untreated) cells.

Figure 13 are bar graphs with a logarithmic axis that shows a graphical representation of calibrated profile data sets for two different samples of whole blood (a) 991116 and (b) 991028 reflecting the biological condition of the cells using a panel of 24 members, each member corresponding to a gene locus, the baseline profile data set being derived from untreated cells. The calibrated data sets for cells exposed for six hours to three inflammation inducing agents (lipopolysaccharide, heat killed staphylococci, and phytohemaglutinin) are compared for each sample. (c) shows a direct comparison of LPS stimulated 991116 with respect to 991028 as the baseline profile data set (d) shows a direct comparison between unstimulated 991116 and 991028.

Figure 14 is a bar graph with a logarithmic axis that shows a graphical representation of calibrated profile data sets using a panel of 22 constituents, each constituent corresponding to a gene locus, the baseline profile data set being derived from untreated cells. Whole blood is exposed for six hours *ex vivo* to three inflammation inducing agents (lipopolysaccharide, heat killed staphylococci, and phytohemaglutinin) which are then treated with a single anti-inflammatory agent (methyl prednisolone) to reveal similarities and differences in the effect of a single agent on cell populations differing in their biological condition.

Figure 15 is a bar graph with a logarithmic axis that shows a graphical representation of calibrated profile data sets for whole blood where one calibrated data set refers to a subject (subject 2) who has been treated *in vivo* with a corticosteroid (dexamethasone), a second data set refers to the treatment of a blood sample from the same subject prior to *in vivo* treatment where that sample has been treated *ex vivo* (*in vitro*) and the third data set refers to a second subject treated *in vivo* with dexamethasone (subject 1). The data sets demonstrate the reproducibility and predictability of an *ex vivo* (*in vitro*) treatment of blood compared to *in vivo* treatment with the same agent. The figure also shows minor variation between samples from different subjects reflecting interpersonal variability. A panel of 14 constituents is provided. The baseline profile data set is derived from untreated whole blood from the cognate subject.

Figure 16 is a bar graph with a logarithmic y axis that shows a graphical representation of calibrated profile data sets for whole blood where one calibrated data set refers to (a) 2 subjects who have been treated *in vivo* with an inactive placebo for 3 days and (b) active prednisolone for 3 days at 100 mg/day. The data set shows some variation between samples from different subjects treated with the same drug. The data sets demonstrate similarity of responses across the same gene loci, as well as, quantitative variation at other loci suggesting quantifiable interpersonal variation. A panel of eight members is provided. The baseline profile data set is derived from untreated whole blood.

Figure 17 is a bar graph with logarithmic y axis that shows a graphical representation of calibrated precision profile data sets for two samples taken from a single subject within a 19 day period using a panel (e.g., inflammation panel) of 24 members where each member corresponds to a unique gene locus. The baseline profile data set relates to peripheral blood taken from the subject prior to treatment.

Figure 18 (a-e) are bar graphs with a logarithmic axis that show a graphical representation of calibrated profile data sets for each of 5 subjects from which a blood sample has been taken. Each of the blood samples was exposed

to the inflammatory agent phytohemagglutin (PHA) or to a therapeutic agent (anti-inflammatory agent) at different concentrations: 0.1μM, 0.3uM, 1μM, 3μM and 5μM, for a 4 hour period *ex vivo* (*in vitro*) so as to determine the optimum dose for treating the subject. A panel of 6 constituents were used corresponding to 6 gene loci. The baseline profile data set was untreated sample obtained from the cognate donor.

Figure 19 is a bar graph with a logarithmic axis that shows a graphical representation of calibrated profile data sets for three different subjects having different biological conditions using a panel with 24 constituents. The profile data sets show variability according to these conditions providing the basis for a diagnostic signature panel. (a) shows a calibrated profile data set for a smoker against a baseline for a non-smoker. (b) shows a calibrated profile data set for a subject with chronic obstructive pulmonary disease against a baseline for a subject lacking this disease. The baseline profile data set is derived from a subject that is "normal" with respect to these conditions.

Figure 20 illustrates that an individual responses can be distinguished from a similarly treated population. A comparison of the response of a single animal compared to its experimental cohort (n=5 animals) with respect to a single locus (GST-P) is provided. The baseline data set is the cohort average. The figures shows that this animal varied significantly from the daily, population average in the first two days of the study, but became more similar to the cohort average with time after treatment with acetaminophen.

Figure 21 is a bar graph with a logarithmic axis that shows a graphical representation of calibrated profile data sets for samples of blood treated *ex vivo* with LPS or LPS and one of three anti-inflammatory herbals (Echinacea, Arnica or Siberian Ginseng) at a concentration of 200 ug/ml. A panel of 24 constituents is used. The baseline profile data set is derived from LPS stimulated cells absent a herbal treatment. The figure illustrates the effectiveness of the use of the calibrated precision profile to investigate the overall effects of complex compounds such as nutraceuticals whose biological effect is a summation of more than one activity. In this case, each of the herbals is consumed as an immunostimulant, however the calibrated precision profiles reveal a unique

pattern shows a mixture of both immunostimulatory and anti-inflammatory effects.

Figure 22 is a bar graph with a logarithmic axis that shows a graphical representation of calibrated profile data sets for samples of blood treated *ex vivo* with LPS or LPS and methylprednisolone or LPS and Arnica. The baseline profile data set is LPS treated blood sample.

Figure 23 is a bar graph with a logarithmic axis that shows a graphical representation of calibrated profile data sets for samples of THP-1 cells treated with LPS or LPS and Arnica at three different concentrations using a panel of 22 constituents. The baseline profile data set is untreated THP-1 cells. The figure illustrates a concentration response with respect to the gene expression across the calibrated profile.

Figure 24 is a bar graph with a logarithmic axis that shows a graphical representation of calibrated profile data sets for samples of THP-1 cells treated *ex vivo* with four different commercial brands of Echinacea using a panel of 8 constituents. The baseline profile data set is untreated THP-1 cells.

Figure 25 illustrates the use of the calibrated profile to compare relative efficacy across brands, or different formulations. Calibrated profile data sets for herbal preparations from different manufacturing sources with respect to an indicator monocytic cell line (THP-1) are shown graphically, the baseline profile data set being THP-1 cells absent the herbal. (a) three commercial herbal Echinacea preparations at 250 (ug/ml); (b) three herbal preparations at different concentrations (250ug/ml, 50ug/ml and 3-10ug/ml) (c) four commercial Echinacea brands at 250 ug/ml).

Detailed Description of Specific Embodiments

As used in this description and the accompanying claims, the following terms shall have the meanings indicated, unless the context otherwise requires:

A "collection of cells" is a set of cells, wherein the set has at least one constituent.

A "population of cells" includes one or more cells. A population of cells may refer to cells *in vivo* or to *in vitro* cultures. *In vitro* cultures may include organ cultures or cell cultures where cell cultures may be primary or continuous cell cultures of eukaryotic or prokaryotic cells. Cell lines can be primary cultures or cell samples, e.g. from a tumor, from blood or a blood fraction, or biopsy explants from an organ, or can be established cell lines or microbial strains.

A "region of the subject" from which proteins are obtained may be (but is not required to be) the same part of the subject from which has been obtained a collection of cells or a population of cells. The cells and the proteins may both be obtained from blood of the subject, for example. Alternatively, for example, the cells may be obtained from blood and the proteins may be obtained from a scraping of tissue or vice versa. Similarly, the proteins may be obtained from urine of the subject, for example, whereas the cells may be obtained elsewhere, as, for example, from blood.

A "panel" of genes is a set of genes including at least two constituents.

A "normative" condition of a subject to whom a composition is to be administered means the condition of a subject before administration, even if the subject happens to be suffering from a disease.

An "expression" of a gene includes the gene product whether messenger RNA or protein resulting from translation of the messenger RNA.

A "large number" of data sets based on a common panel of genes is a number of data sets sufficiently large to permit a statistically significant conclusion to be drawn with respect to an instance of a data set based on the same panel.

A "biological condition" of a subject is the condition of the subject in a pertinent realm that is under observation, and such realm may include any aspect of the subject capable of being monitored for change in condition, such as health, disease including cancer; trauma; aging; infection; tissue degeneration; developmental steps; physical fitness; obesity, or mood. As can be seen, the conditions may be chronic or acute or simply transient. Moreover, a targeted biological condition may be manifest throughout the organism or population of

cells or may be restricted to a specific organ (such as skin, heart, eye or blood). The term "biological condition" includes a "physiological condition".

The "blind administration" of a selected one of a composition or placebo to a subject in a clinical trial involves administering the composition or placebo to the subject in accordance with a protocol pursuant to which the subject lacks knowledge whether the substance administered is the composition or a placebo.

An "organism" is any living cell including microorganisms, animals and plants. An animal is commonly in this context a mammal, but may be a vertebrate non-mammal, as e.g., a zebra fish, or an invertebrate, as, e.g. *Caenorhabditis elegans*.

An "agent" is a composition or a stimulus. A "stimulus" may include, for example ultraviolet A or B, or light therapy for seasonal affective disorder, or treatment of psoriasis with psoralen or treatment of melanoma with embedded radioactive seeds, other radiation exposure, etc. A "composition" includes a chemical compound, a nutraceutical, a combination of compounds, or a complex mixture.

A "clinical indicator" is any physiological datum used alone or in conjunction with other data in evaluating the physiological condition of a collection of cells or of an organism. This term includes pre-clinical indicators.

A "signature panel" is any panel representing a subclass of constituents where the subclass of constituents is selected according to the relatively high level of information concerning a biological condition imparted by each member of the data set.

"Distinct RNA or protein constituent" in a panel of constituents is a panel that includes at least one of RNA and protein and each constituent of the panel is distinct.

A preferred embodiment of the invention is the formation of calibrated data sets that describe a biological condition or an effect of an agent on a biological condition. A calibrated data set represents a set of values that correspond to variations in gene expression where the variations are informative. This approach does not require comprehensive analysis of all gene expression in target cells associated with a particular condition. Nor is any one

single gene locus necessarily of particular significance. Rather a pattern of variation (a profile) is sought that correlates, in a reproducible manner, with a particular condition. There may be no *a priori* knowledge of a correlation but rather a correlation may be established by evaluating a panel of constituents of reasonable size (for example up to 100 constituents) and iteratively testing the gene expression profiles for different subjects or for the same subject from which the most informative loci for a particular condition may be selected. An informative subgroup of constituents in a panel may be selected that consistently vary for a particular condition and this subgroup may then become the signature panel, the signature panel giving rise to a signature profile.

In further embodiments of the invention, any calibrated data set for an individual that has more members than reflective of a single signature panel may be mined for calibrated profiles that correspond to additional signature panels thereby potentially providing new insights into mechanisms of action of a biological condition on sets of genes. Measurement of changes in transcribed RNA in a cell as a result of an environmental change or aging is an exquisitely sensitive measure of the response of a cell. Techniques available today to quantify transcribed RNA in a cell add to the sensitivity of the approach. The preferred embodiments of the invention, which are directed to patterns of change in amounts of transcribed RNA, provide a means to focus and interpret this rich information.

In contrast to the above approach, much attention in the prior art has been directed to the sequencing of the human genome and the identification of all the genes encoded therein. Accompanying the growing amount of sequence data, microarrays provide a means to survey thousands of gene sequences for mutations. Microarrays are being used to provide DNA profiles that identify mutations in an individual and those mutations will be associated with predictions concerning development of disease in those individuals. Transcriptomics and proteomics is now the focus of increasing attention. These studies are directed to analyzing the entire body of RNA and protein produced by living cells. Microarrays provide a method for analyzing many thousands of different human RNAs as to whether they are expressed and by which cells. For

example, a project undertaken by the National Cancer Institute and others to examine mRNAs produced by various types of cancer cells, have revealed 50,000 genes that are active in one or more cancers. The goal of these studies is to identify novel cancer drugs that are directed to knocking out or enhancing the production of certain proteins. (Kathryn Brown, The Human Genome Business Today, Scientific American, July 2000, p.50; Julia Karow, The "Other" Genomes, Scientific American, July 2000, p.53; Ken Howard, "The Bioinformatics Gold Rush, Scientific American, July 2000, p.58; Carol Ezzell, Beyond the Human Genome, Scientific American, July 2000, p.64; all incorporated by reference). Major efforts in correlating genetic variation of individuals and the functional interrelationships of genes in health and disease are being conducted in a variety of consortia including the single nucleotide polymorphism consortium and the Human Epigenome Consortium (Beck et al. Nature BioTechnology 17 (1999) p 1144). The Epigenome Consortium plans to analyze sets of genome fragments from both healthy and diseased individuals in the 500 different human tissues (Bioworld International: December 22, 1999). This approach seeks to correlate absolute expression of genes associated with a particular condition with the presence of that condition. Examples of prior art that seek to measure gene expression in absolute amounts including by subtractive methods or by determining amounts with respect to housekeeping genes or by targeting a single gene expression system are U.S. 5,643,765; U.S. 5,811,231; U.S. 5,846,720; U.S. 5,866,330; U.S. 5,968,784; U.S. 5,994,076; WO 97/41261; WO 98/24935; WO 99/11822; WO 99/44063; WO 99/46403; WO 99/57130; WO00/22172 and WO00/11208.

We have taken a different and novel approach to the above by identifying reproducible patterns of variation of gene expression that are informative by virtue of the degree of variation between a sample and a baseline for example a subject with the condition and the subject that lacks the condition. The variations may be correlated with other non- genetic indications such as clinical indicators (for humans) of a traditional nature but are not required per se to be causative. Accordingly, the amount of gene expression product (for example RNA transcript) produced by a gene locus in a cell under certain circumstances

is measured and then stored as a value in a first profile data set. This value is calibrated with respect to a second value (a baseline profile data set) to provide a member of a calibrated profile data set. The values recorded for the profile data set, relying on a particular baseline data set to produce a calibrated data set become part of the descriptive record any or all of which can be stored in a database which may be accessed through a global network such that any new data in the form of a profile data set or a calibrated profile data set measured at any global location can be directly compared to an archive of descriptive records including calibrated profile data sets and baseline data sets so as to extend the stored library of profiles and provide predictive or diagnostic data about a particular biological condition or agent.

We have exemplified the use of selected panels of constituents corresponding to gene loci from which quantitative gene expression is measure by for example quantitatively measuring the transcribed RNA in a sample of a subject, for applications that include: (a) measurement of therapeutic efficacy of natural or synthetic compositions or stimuli that may be formulated individually or in combinations or mixtures for a range of targeted physiological conditions; (b) predictions of toxicological effects and dose effectiveness of a composition or mixture of compositions for an individual or in a population; (c) determining how two different agents administered in a single treatment might interact so as to detect any of synergistic , additive, negative, neutral of toxic activity (d) performing pre-clinical and clinical trials by providing new criteria for pre-selecting subjects according to informative profile data sets for revealing disease status and conducting preliminary dosage studies for these patients prior to conducting phase 1 or 2 trials. Gene expression profiling may be used to reduce the cost of phase 3 clinical trials and may be used beyond phase 3 trials; (e) labeling for approved drugs; (f) selection of suitable medication in a class of medications for a particular patient that is directed to their unique physiology; (g) diagnosing or determining a prognosis of a medical condition or an infection which may precede onset of symptoms or alternatively diagnosing adverse side effects associated with administration of a therapeutic agent; (h)

managing the health care of a patient; and (i) quality control for different batches of an agent or a mixture of agents.

The Subject

The methods herein can be applied to a subject that includes any living organism where a living organism includes a prokaryote such a bacterium or a eukaryote including single celled eukaryotic organisms at one end of the spectrum and humans at the other and everything in between including plants. The figures relate to calibrated profile data sets obtained from humans and mammals. Nonetheless, the methods disclosed here may be applied to cells of other organism without the need for undue experimentation by one of ordinary skill in the art because all cells transcribe RNA and it is know in the art how to extract RNA from all types of cells.

A tissue sample might include a single cell or multiple cells or fragments of cells. Body fluid includes blood, urine, spinal fluid, lymph, mucosal secretions, hemolymph or any other body fluid known in the art for a subject. For an animal subject, a tissue or fluid sample may be obtained by means of a biopsy needle aspirate, a lavage sample, scrapings and surgical incisions or other means known in the art.

Panels

Steps in selecting constituents in a panel include searching publicly available medical literature for RNA or proteins or sets of RNAs or proteins that directly or indirectly vary with a particular biological condition. A panel containing up to 100 constituents may be selected. According to the condition being examined, just a small subset of the panel constituents may be informative. In determining membership of the panel of genes, it is not necessary for the panel to be an exhaustive selection. Rather it is desired to obtain from the panel an expression profile that discriminates consistently with respect to the targeted physiological or biological condition. Moreover, a panel is not necessarily selected according to an expected profile of gene expression in cells that directly respond to a biological effect. For example, gene expression associated with liver metabolism may be analyzed in a blood sample. Figures 20

and 22 provide calibrated profiles of whole blood treated with herbal agents using markers for liver metabolism.

The number of constituents in a panel can vary. According to the examples provided below, panels of up to 24 genes are selected for evaluating expression levels. Although a panel may be as large as 100 constituents, it is desirable for a particular panel to have no more than 24 constituents, more particularly, less than 12 constituents. For example, subsets of no more than 8 genes have been used that may be derived from a larger panel but which are sufficiently informative to effectuate discrimination. The number of constituents in a panel for which expression is monitored may vary widely depending on the context. For example, Figure 1 describes data acquisition from *in vitro* cell culture and from animal toxicology studies, which includes expression of about 25 to 100 or more genes. In contrast, selection of markers or surrogate markers include for example three to 100 genes, preferably five to 50 or five to 25 genes to be analyzed from samples obtained in clinical studies. In this manner markers or surrogate markers having predictive value for a medical condition, such as a genetic predisposition, a response to therapeutic agent, an inflammatory condition, or an infection, etc. can be identified and cumulatively larger populations can be obtained to refine the correlations. A health profile can then be generated for an individual subject using a low volume blood sample. The blood sample can be analyzed for expression profile data of about 100 - 500 genes, comprising markers or surrogate markers of a number of medical conditions (Fig. 1: right panel). Panels of varying sizes may be utilized as necessary and subsequent refinements in methodology may lead to selection of subsets having panels as large as 15 genes or 12 genes or as small as 6, 5, 4, 3 or 2 genes.

It is envisaged that any single biological condition may be described by a signature panel having a small number of highly informative constituents providing a signature calibrated profile (also referred to as a fingerprint). The presence of highly informative loci is demonstrated in several of the accompanying figures. For example, Figure 11(a) Il-2, Il-4 and Il-5 appeared to be highly informative. Highly informative constituents in Figure 21 include the

interleukins. The signature panel may provide a signature profile or fingerprint which is sufficiently robust to serve as a standard in describing a particular biological condition or an effect of a particular agent on a biological condition.

For purposes of illustrating a signature panel, constituents of a panel for measuring inflammation have been provided that are informative with respect to a particular biological condition. For example, we have used a panel for inflammation that has 6 constituents- Il-1a, Il-6, Il-8, Il-18, GMCSF and IFN-g in Figure 18(a)–(e) to determine the response of 5 subjects to varying concentrations of drugs. This group of constituents is a subset of a larger panel of inflammation related gene loci such as shown in Figure 19a and Figure 19b where the Inflammatory Panel includes Il-a, Il-b, Il-2, Il-3, Il-4, Il-6, Il-7, Il-8, Il-10, Il-12p40, Il-15, Il-15, Il-18, GM-CSF, Ifn-gamma, TGF-b, cox-2, ICE, MMP-9, ICAM, TNF-a and TNF-b. The subset of constituents were selected on the basis of the information sought concerning the biological condition.

Embodiments of the invention provide examples of at least 4 different panels which may be used separately or together. These panels are an inflammatory panel (TNF-a, Il-1b, ICAM, Il-8, Il-10, Il-12p40, ICE, cox-2, cox-1 and mmp-3) a cell growth and differentiation panel (c-fos, c-jun and STAT3), a toxicity panel (SOD-1, TACE, GR, HSP70, GST, c-fos, c-jun, INOS) and a liver metabolism panel (INOS, cyp-a and u-pa). Other panels include skin response or prostate cancer or endothelial/cardiovascular response panels or cell growth or differentiation or liver metabolism panels. Although provided as examples, the above panels are not intended to be limiting.

Gene Expression

For measuring the amount of a particular RNA in a sample, we have used methods known to one of ordinary skill in the art to extract and quantify transcribed RNA from a sample with respect to a constituent of a panel. RNA is extracted from a sample such as a tissue, body fluid, or culture medium in which a population of a subject might be growing. For example, cells may be lysed and RNA eluted in a suitable solution in which to conduct a DNAse reaction. First strand synthesis may then performed using a reverse transcriptase. Gene amplification, more specifically quantitative PCR assays,

can then conducted and the gene of interest size calibrated against a marker such as 18S rRNA (Hirayama et al., Blood 92, 1998: 46-52). Samples are measured in multiple duplicates for example, 4 replicates. Relative quantitation of the mRNA is determined by the difference in threshhold cycles between the size marker and the gene of interest. In an embodiment of the invention, quantitative PCR is performed using amplification, reporting agents and instruments such as those supplied commercially by PE Biosystems (Foster City, CA). Given a defined efficiency of amplification of target transcripts, the point (e.g., cycle number) that signal from amplified target template is detectable may be directly related to the amount of specific message transcript in the measured sample. Similarly, other quantifiable signals such as fluorescence, enzyme activity, disintegrations per minute, absorbance, etc., when correlated to a known concentration of target templates (e.g., a reference standard curve) or normalized to a standard with limited variability can be used to quantify the number of target templates in an unknown sample.

Although not limited to amplification methods, quantitative gene expression techniques may utilize amplification of the target transcript. Alternatively or in combination with amplification of the target transcript, amplification of the reporter signal may also be used. Amplification of the target template may be accomplished by isothermic gene amplification strategies, or by gene amplification by thermal cycling such as PCR. It is desirable to obtain a definable and reproducible correlation between the amplified target or reporter and the concentration of starting templates.

It is envisaged that techniques in the art using microfluidics for example and highly sensitive markers will enable quantitation of RNA to occur directly from a single cell or lysed cell. This may rely on amplification of a marker but may not require amplification of the transcripts themselves. The amount of transcript measured for any particular locus is a data point or member of the first profile data set for a particular panel.

According to embodiments of the invention, a first profile data set is derived from the sample, the first profile data set including a plurality of members, each member being a quantitative measure of the amount of a RNA

transcribed from a gene locus, the gene locus being a constituent in a panel of constituents. A first profile data set may be obtained from a quantitative measure of the amount of a distinct RNA or protein corresponding to a gene locus. The figures provided here are directed to RNA. However, the method could be applied using proteins where sensitive quantitative techniques are available for measuring the amount of a distinct protein in a cell.

Baseline Profile Data Sets

The analyses of samples from single individuals and from large groups of individuals provide a library of profile data sets relating to a particular panel or series of panels. These profile data sets may be stored as records in a library for use as baseline profile data sets. As the term "baseline" suggests, the stored baseline profile data sets serve as comparators for providing a calibrated profile data set that is informative about a biological condition or agent. It is anticipated that many baseline profile data sets will be stored in libraries and classified in a number of cross-referential ways. One form of classification might rely on the characteristics of the panels from which the data sets are derived. Another form of classification might be the use of a particular biological condition. The concept of biological condition encompasses any state in which a cell or population of cells might be at any one time. This state might reflect geography of samples, sex of subjects or any other discriminator. Some of the discriminators may overlap. The libraries might also be accessed for records associated with a single subject or particular clinical trial. The classification of baseline profile data sets may further be annotated with medical information about a particular subject, a medical condition, a particular agent etc.

The choice of a baseline profile data set for creating a calibrated profile data set is related to the biological condition to be evaluated, monitored, or predicted, as well as, the intended use of the calibrated panel, e.g., as to monitor drug development, quality control or other uses. It might be desirable to access baseline profile data sets from the same subject for whom a first profile data set is obtained or from different subject at varying times, exposures to stimuli,

drugs or complex compounds; or may be derived from like or dissimilar populations.

The profile data set may arise from the same subject for which the first data set is obtained, where the sample is taken at a separate or similar time, a different or similar site or in a different or similar physiological condition. For example, Figure 5 provides a protocol in which the sample is taken before stimulation or after stimulation. The profile data set obtained from the unstimulated sample may serve as a baseline profile data set for the sample taken after stimulation. The baseline data set may also be derived from a library containing profile data sets of a population of subjects having some defining characteristic or biological condition. The baseline profile data set may also correspond to some *ex vivo* or *in vitro* properties associated with an *in vitro* cell culture. The resultant calibrated profile data sets may then be stored as a record in a database or library (Figure 6) along with or separate from the baseline profile database and optionally the first profile data set although the first profile data set would normally become incorporated into a baseline profile data set under suitable classification criteria.

Selected baseline profile data sets may be also be used as a standard by which to judge manufacturing lots in terms of efficacy, toxicity, etc. Where the effect of a therapeutic agent is being measured, the baseline data set might correspond to gene expression profiles taken before administration of the agent. Where quality control for a newly manufactured product is being determined, the baseline data set might correspond with a gold standard for that product. However, any suitable normalization techniques may be employed. For example, an average baseline profile data set is obtained from authentic material of a naturally grown herbal nutraceutical and compared over time and over different lots in order to demonstrate consistency, or lack of consistency, in lots of compounds prepared for release.

Calibrated Data

A calibrated profile data set may be described as a function of a member of a first profile data set and a corresponding member of a baseline profile data set for a given gene locus in a panel. For example, calibrated profile data sets

may be derived by calculating a ratio of the amount of RNA transcribed for a panel constituent in a cell sample in an environmental including intervention such as a therapeutic treatment or at a particular time (first profile data set) with respect to the amount of RNA transcribed for the same panel constituent in a cell that differs in some manner from the sample (baseline profile data set) (Figures 5 and 6). We have found that calibrated profile data sets to be reproducible in samples that are repeatedly tested (Figure 17). We have also found that calibrated profile data sets obtained when samples from a subject are exposed *ex vivo* to a compound are comparable to calibrated profile data from a sample that has been exposed to a sample *in vivo* (Figure 14 and Figure 16(a),(b)). We have also found that an indicator cell line treated with an agent can provide comparable calibrated profile data sets to those obtained from *in vivo* or *ex vivo* populations of cells (Figure 15). Moreover, we have found that administering a sample from a subject onto indicator cells can provide informative calibrated profile data sets with respect to the biological condition of the subject including the health, disease states, therapeutic interventions, aging or exposure to environmental stimuli or toxins of the subject (Figure 25).

A preferred use of a calibrated profile data set is to evaluate a biological condition of a subject. This may be for purposes of diagnosis or prognosis of a clinical disorder. It is desirable to obtain a calibrated data set that describes a state of health or alternatively a state of age or body mass or any condition or state that an individual subject might find themselves to be in. For example, the biological condition might relate to physical activity, conditioning or exercise, mental state, environmental factor such as medication, diet, or geography or exposure to radiation or environmental contamination or infectious agent, biological or environmental toxin. If health or conversely a clinical disorder is being evaluated, calibrated profiles data sets may be used for monitoring change in health status by periodic or regular comparison of profiles; the disorder may be a complex disease process possibly involving multiple gene including inflammation, autoimmune disease, degenerative disease, allergy, vascular disease, ischemia, developmental disease, hormonal conditions and infectious diseases. The clinical disorder may further include arthritis, asthma, multiple

sclerosis and perimenopausal changes. The biological condition may affect a system of a subject including a respiratory, vascular, nervous, metabolic, urinary, reproductive, structural and immunological system or other metabolic state. The above examples of a biological condition are given by way of illustration and are not intended to be limiting.

Similarly, calibrated profile data sets can be used to measure, monitor or predict the host response to an infectious agent for purposes of identifying the infectious agent, assessing the duration of infection, the extent of exposure or making therapeutic decisions.

The evaluation of activity of an agent may require a series of calibrated profiles. It is here shown that calibrated profile data sets can be used to describe the biological activity of an agent that might be a single compound or a complex compound such as a nutraceutical or herbal. The agent can be assayed using indicator cells, *ex vivo* cell populations or by *in vivo* administration. These assays may rely on a series of signature panels or enlarged panels for different biological conditions. The resultant calibrated profiles may then be used to infer likely *in vivo* activity from the *in vitro* study. Insights into toxicity and mechanisms of action can also be inferred from calibration profile data sets. For example, the herbal Echinacea is believed to have both immunostimulatory and anti-inflammatory properties although neither has been measured systemmatically. We have provided a systematic approach to investigate the biological activities of these and other herbs. We investigated the alleged immunostimulatory properties of the herbs by comparing the effect of treating the indicator cell line THP-1 or peripheral blood cells with the agent to untreated cells. Untreated cells include LPS stimulated untreated cells. Untreated cells were used as a baseline profile data set to measure the difference in gene expression between a baseline profile data set and the experimental treatment with the compound. Baseline profile data sets included a single sample or an average value from a series of experiments. The resultant calibrated profile data sets could then be compared with a library of calibrated profile data sets for a particular herb or/and libraries associated with different agents or conditions.

From the information obtained about a previously undescribed agent, a signature panel may be derived optionally together with a signature profile to serve as a gold standard for testing other batches of the same agent.

Calculation of Calibrated Profile Data Sets and Computational Aids

The function relating the baseline and profile data sets is in a preferred embodiment, a ratio expressed as a logarithm. The calibrated profile data set may be expressed in a spreadsheet or represented graphically for example, in a bar chart or tabular form but may also be expressed in a three dimensional representation. Preferably the constituent is itemized on the x-axis and the logarithmic scale is on the y-axis. Members of a calibrated data set may be expressed as a positive value representing a relative enhancement of gene expression or as a negative value representing a relative reduction in gene expression with respect to the baseline.

Each member of the calibrated profile data set should be reproducible within a range with respect to similar samples taken from the subject under similar conditions. For example, the calibrated profile data sets may be reproducible within one order of magnitude with respect to similar samples taken from the subject under similar conditions. More particularly, the members may be reproducible within 50% more particularly reproducible within 20%. Each member of the calibrated profile data set has a biological significance if it has a value differing by more than an amount D, where D=F(1.1)-F(.9) and F is a second function.

It is the pattern of increasing, decreasing and no change in gene expression from the plurality of gene loci examined in the panel that is used to prepare a calibrated profile set that is informative with regards to a biological condition, biological efficacy of an agent treatment conditions or for comparison to populations and which may be used to identify likely candidates for a drug trial, used in combination with other clinical indicators to be diagnostic or prognostic with respect to a biological condition or may be used to guide the development of a pharmaceutical or nutraceutical through manufacture, testing and marketing.

The numerical data obtained from quantitative gene expression and numerical data from calibrated gene expression relative to a baseline profile data set may be stored in databases or digital storage mediums and may retrieved for purposes including managing patient health care or for conducting clinical trials or for characterizing a drug. The data may be transferred in networks via the World Wide Web, email, or internet access site for example or by hard copy so as to be collected and pooled from distant geographic sites (Figure 8).

In a preferred embodiment, a descriptive record is stored in a single or multiple databases where the stored data includes the raw gene expression data (first profile data set) prior to transformation by use of a baseline profile data set, as well as a record of the baseline profile data set used to generate the calibrated profile data set including for example, annotations regarding whether the baseline profile data set is derived from a particular signature panel and any other annotation that facilitates interpretation and use of the data.

Because the data is in a universal format, data handling may readily be done with a computer. The data is organized so as to provide an output optionally corresponding to a graphical representation of a calibrated data set. For example, a distinct sample derived from a subject being at least one of RNA or protein may be denoted as P_1. The first profile data set consists of M_j where Mj is a quantitative measure of a distinct RNA or protein constituent. The record R_i is a ratio of M and P and may be annotated with additional data on the subject relating to for example, age, diet, ethnicity, gender, geographic location, medical disorder, mental disorder, medication, physical activity, body mass and environmental exposure. Moreover, data handling may further include accessing data from a second condition database which may contain additional medical data not presently held with the calibrated profile data sets. In this context, data access may be via a computer network.

The above described data storage on a computer may provide the information in a form that can be accessed by a user. Accordingly, the user may load the information onto a second access site including downloading the information. However, access may be restricted to users having a password or

other security device so as to protect the medical records contained within. A feature of this embodiment of the invention is the ability of a user to add new or annotated records to the data set so the records become part of the biological information.

The graphical representation of calibrated profile data sets pertaining to a product such as a drug provides an opportunity for standardizing a product by means of the calibrated profile, more particularly a signature profile. The profile may be used as a feature with which to promote the drug.

The various embodiments of the invention may be also implemented as a computer program product for use with a computer system. The product may include program code for deriving a first profile data set and for producing calibrated profiles. Such implementation may include a series of computer instructions fixed either on a tangible medium, such as a computer readable media (for example, a diskette, CD-ROM, ROM, or fixed disk), or transmittable to a computer system via a modem or other interface device, such as a communications adapter connected to a network over a medium. The medium may be either a tangible medium (for example, optical or analog communications lines) or a medium implemented with wireless techniques (for example, microwave, infrared or other transmission techniques). The series of computer instructions preferably embodies all or part of the functionality previously described herein with respect to the system. Those skilled in the art should appreciate that such computer instructions can be written in a number of programming languages for use with many computer architectures or operating systems. Furthermore, such instructions may be stored in any memory device, such as semiconductor, magnetic, optical or other memory devices, and may be transmitted using any communications technology, such as optical, infrared, microwave, or other transmission technologies. It is expected that such a computer program product may be distributed as a removable medium with accompanying printed or electronic documentation (for example, shrink wrapped software), preloaded with a computer system (for example, on system ROM or fixed disk), or distributed from a server or electronic bulletin board over the network (for example, the Internet or World Wide Web). In addition, a

computer system is further provided including derivative modules for deriving a first data set and a calibration profile data set.

Clinical Trials

The use of calibrated profile data sets for performing clinical trials is illustrated in Figure 10 using the above-described methods and procedures for running a clinical trial or managing patient care. Moreover, standardization between laboratories may be achieved by using a particular indicator cell line such as THP-1 which is stimulated by a known stimulator such as lipopolysaccharide so that resultant profile acts as a measure that the laboratory is performing the protocol correctly.

Examples of how embodiments of the invention may be used for augmenting clinical trials includes providing new methods for patient selection. Clinical trials in which candidate subjects are included or excluded according to a predetermined optimum calibrated profile for a given biological condition can result in more precise monitoring than would be otherwise possible. It can also result in a greater efficiency in clinical trial design because unsuitable patients that have, for example, complicating factors or conditions can be screened out. The calibrated profile data will also enhance the "signal to noise" by removing non-responders from double blind placebo studies. The basic structure of a clinical trial design using gene expression profiling could follow any of several formats. These include testing body fluid from a candidate patient in the trial *ex vivo* against a new therapeutic agent and analyzing the calibrated profiles with respect to an agent-treated and placebo-treated samples using a predetermined panel and evaluating whether the candidate patient would be likely to respond without adverse effects to the composition being tested. In selected indications, profile data obtained from *in vitro* cell cultures or organ cultures may be desired where the cell originates from a target subject or from another subject or from an established cell line, or from a cell samples removed from the target subject where the cell samples may be obtained from any body fluid including a blood, urine, semen, amniotic, or a cerebrospinal fluid sample, or from a scraping from mucosal membranes such as from the buccal cavity, the eye, nose, vagina or by means of a biopsy including epithelial, liver, sternum marrow, testicular, or

from tumor tissue removed surgically from a tumor at any location. The above-described sources of samples are applicable to any medical use in which calibrated profile data sets are desired.

In vitro dosage and toxicity studies using calibrated profile data sets obtained from indicator cell lines or samples of the patient tested *ex vivo* can provide useful information prior to initiation of the clinical trial and can significantly reduce the cost and time of a clinical trial while increasing the likelihood of identifying the presence of beneficial effect. In particular, the dose can be optimized on an individualized basis to maximize the impact on therapeutic outcome. For example, Figure 12 shows how *ex vivo* blood cells respond to the stimulatory effect of LPS and the subsequent treatment with an anti-inflammatory drug (methotrexate, meclofenamate or methylprednisolone). The data show how the effect of methotrexate and meclofenamate generates similar calibrated profile data sets where the baseline is LPS treated blood. In contrast, the methylprednisolone has a substantially different effect from the other two compounds. A similar type of analysis can be performed with complex mixtures as illustrated in Figure 21 in which the calibrated profiles obtained when Echinacea, Arnica and Siberian Ginseng applied to LPS stimulated blood *ex vivo* are compared. In this example, all three agents appear to act differently from each other with respect to a sample from a single subject. Similar analyses can be used to compare compounds with unknown targets or activities or metabolic patterns to compounds, complex or simple, with known or pre-determined profiles.

The above methods and procedures can be utilized in the design and running of clinical trials or as a supplemental tool. Moreover, the above methods and procedures can be used to monitor the patients' health as well as the patient's responsiveness to an agent before during and after the clinical trial. This includes monitoring whether multiple agents interfere with each other, act synergistically or additively or are toxic or neural with respect to each other. This type of information is very important as individuals take an increasing number of medications.

Similarly, the methods and procedures described above may be used to manage patient care for an individual or a population. Such methods and procedures may also be used to develop a regional or global research network that uses calibrated profile data sets and the resulting databases to conduct research or trials.

Both the calibration profile data sets in graphical form and the associated databases together with information extracted from both are commodities that can be sold together or separately for a variety of purposes. For example, graphic representations of calibration profile data sets can provide a description of a product with respect to its activity that may be used to promote the product. Alternatively, the graphical form of the calibrated profile data sets and access to baseline profile databases provide a means for manufacturers to test discrete batches of product against a gold standard.

The data can be used strategically for design of clinical trials. It can also be useful for physicians practicing at remote sites to offer personalized healthcare to a patient. Accordingly, the physician might set up personalized databases for calibrated profile data sets prior to and after treatment of a particular condition. New data on the subject could be added to the personalized database at each visit to the doctor. The data could be generated at remote sites by the use of kits that permit a physician to obtain a first profile data set on a sample from a patient. For remote users to access the site, it is envisaged that secured access to the global network containing libraries of baseline profile data sets and calibrated profile data sets, classified by particular criteria and representing data from larger populations than a single individual, would be necessary. The access to the global database may be password protected thereby protecting the database from corrupted records and safeguarding personal medical data. The graphical form provided by the calibrated data sets may be used to create catalogs of compounds in a pharmacopiae complete with toxic effects that might arise for particular individuals as well as other types of drug interactions.

Access to the global data base may include the option to load selected data onto a second access site. This process could include downloading the

information to whatever site is desired by the user and could include securing hard copies of information. It is desirable to control how and what data is offloaded or dopied to maintain the integrity of the database. It is envisaged that while a global network of clinical data would be an informational resource, it would have utility is conducting research that might include epidemiological studies and studies concerning the mechanism of action of an agent and studies concerning the nature of interpersonal variability as determined by calibrated profile data sets.

Examples of Medical Uses

(a) Early detection of infectious diseases. Markers or surrogate markers from mice may be obtained for measuring gene expression in humans that indicate early or immediate response to infection, for example, to a virus such as hepatitis virus, or to a bacterium such as *Mycobacterium tuberculosis* (the Gram-positive etiologic agent of tuberculosis) (see Figure 4). Candidate genes are identified and changes in expression of those genes in the presence of a challenge provide a set of markers. The set of markers can combine markers encoded by the genome of the subject and one more distinctive markers encoded by the genome of the infectious agent. For example, changes in expression of an immediate early gene of a virus, e.g. a gene encoding an enzyme of viral replication, and a host gene such as the gene for any or all of IL-2, IL-4 and IL-5, can comprise markers or surrogate markers for a medical condition capable of detecting that condition prior to the onset of medical symptoms. This method affords earlier detection of an infection than is possible using current diagnostic techniques.

(b) Toxicity profiles and mechanistic profiles obtained from an *in vitro* assay and *in vivo* assays. Toxicity and mechanistic information arising from the administration of a compound to a population of cells can be monitored using calibrated profile data sets. The following is an example of an experimental protocol for obtaining this information. Firstly, an experimental groups is established: (1) control cells maintained without therapeutic agent and without stimulus; (2) cells treated with therapeutic agent but without stimulus; (3) cells without therapeutic agent but with stimulus, (4) sample with therapeutic agent

and with stimulus. The population of cells can be selected from primary cell cultures prepared in culture plates using methods well established in the art; or mature differentiated cell preparation from whole blood or isolated monocytes from the target organism, which in this example is mouse.

The cells are stimulated so as to present a targeted physiological condition by pretreatment with LPS purified from a Gram-negative bacterium (a variety of LPS preparations from pathogenic bacteria, for example, from *Salmonella typhimurium* and from *Escherichia coli* O1157:H7, are available from Sigma, St. Louis, MO). The therapeutic agent administered to the cell samples in this example is an inhibitor of an enzyme known to be key in disease etiology, namely an inhibitor of a protease or a nucleic acid polymerase. Following treatment by addition of the therapeutic agent and further incubation for four to six hours, samples of the cells are harvested and analyzed for gene expression. Nucleic acid, specifically RNA, can be prepared from the sample by methods known to one or ordinary skill in the art (see, for example, the Lyse-N-Go™ reagent, Pierce Chem. Co:, Rockford, IL). Samples are analyzed by QPCR according to a quantitative replicative procedure, (quantitative polymerase chain reaction procedure (QPCR)) (see, for example, Gibson,U. 1996 Genome Res.6:995-1001, and references cited therein). Total RNA was assessed using universal primers. Toxicity of the agent for cells can be measured in untreated cells by vital stain uptake, rate of DNA synthesis (autoradiography of labeled nucleic compared to cells stained), stain by DNA-specific eyes (Hoechst), etc. Mechanistic profiles can be determined by analysis of the identifies of *de novo* up- or down-regulated genes. Further, in the presence of a therapeutic agent, some genes are not expressed, indicating potential efficacy of the therapeutic agent in suppressing the effects of stimulation by the LPS. For example, in Figure 21, levels of ICE that are somewhat stimulated in the presence of LPS +Echinacea are substantially depressed by LPS + Arnica relative to LPS stimulated cells absent agent. Levels of HSP 70 which are depressed in the presence of LPS+Echinacea are substantially stimulated in the presence of LPS +Arnica, and LPS + Siberian Ginseng relative to LPS stimulated cells absent the addition of an agent. Levels of IL-12p40 which are slightly increased in the

presence of LPS + Echinacea are substantially depressed in the presence of LPS + Arnica and LPS +Siberian Ginseng relative to LPS stimulation. In contrast to the above using nutraceuticals, Figure 16 shows a much enhanced reduction of gene expression in whole blood for IL-1a, Il-1b, Il-7, Il-10, IL-IL-15, IFN-g, TGF-b, TNF-b cox-2, and ICAM in the presence of prednisolone +LPS when compared to arnica +LPS or nothing +LPS.

(c) Quantitation of gene expression in a blood cell to predict toxicity in another tissue or organ.

Leukocytes can be obtained from a blood sample of a subject, for the purpose of assessing the appearance of a pathological condition in another organ, for example, the liver. A profile data set is obtained of genes expressed in the leukocytes, for example, genes encoding a set of lymphokines and cytokines. The data set is compared to that of the database, to examine correlations for example to other subjects, and to the subject prior to administration of a therapeutic agent.

By this method, a correlation can be drawn between, for example, administration of acetaminophen (Tylenol) and sensitivity to this therapeutic agent and manifested by liver damage. An early prediction of therapeutic agent sensitivity, detected prior to the onset of actual damage to the liver, can be clinically available so that the subject receives no further administration of acetaminophen. The success of the database is the ability to detect a correlation or correlations prior to the onset of traditional medical assessments, such as increase in bilirubin level or other indication of liver pathology.

(d) Calibrated profiles from blood cells for prognosis of severity and prediction of adverse reactions in treatment of an autoimmune disease.

The probability and timing of onset of symptoms of an autoimmune disease, for example, rheumatoid arthritis, may be monitored by appearance of expression of markers or surrogate markers as determined by the methods of gene expression profiling of markers or surrogate markers and comparison to a profile database as described above. Thus an indication of imminent onset can be obtained, and advance management by utilization of preventive measures to forestall onset, can be taken. Further, the user can choose a set of potential

therapeutic agents, and assess for a given agent, the probability that a subject will present an adverse reaction if given a full course of treatment, prior to that full course. For example, using embodiments of the invention, a single dose of the agent methotrexate can be administered to a subject having arthritis and in need of a therapeutic agent. If the gene expression profile data set of the subject in response to a single dose of methotrexate correlates with data sets from subjects having adverse reactions to this agent, then administration of a full course of methotrexate is counterindicated. Conversely, if the gene expression profile data set correlates with those of subjects who have responded positively to administration of a course of methotrexate treatment, then this therapeutic agent can be administered to the subject with much lower probability of adverse reaction.

Discussion of Figures

Figures 1-4 illustrate some of the applications of calibrated profile data sets. In Figure 1, three possible scenarios are provided. Firstly, a candidate therapeutic agent may be tested to determine its molecular pharmacology and toxicology profiles. The test might include obtaining calibrated profile data sets for a series of panels selected on the basis of what activity is predicted for the drug. The population of cells exposed to the agent may be the result of *in vivo* administration as depicted by the mouse or direct exposure *in vitro* where the cells may be an indicator cell line or an *ex vivo* sample from the subject. The result of the screen is the identification of more effective drug candidates for testing in human subjects.

The second scenario in Figure 1 is the use of calibrated profile data sets to identify a suitable clinical population for screening a potential therapeutic agent. Both demonstration of lack of toxicity and demonstration of clinical efficacy require certain assumptions about the clinical population. The calibrated profile data sets provides a means for establishing those assumptions with respect to the biological condition of the individuals selected for the clinical trials.

The third scenario in Figure 1 is the opportunity to practice individualized medicine which may include creating an archive of calibrate profile data sets on the individual in a state of health such that changes can be

identified using signature panels so as to permit prognosis or diagnosis of a particular condition. Moreover, stored information about the patient in the form of calibrated profile data sets permits selecting one of a group of possible therapeutic agents most like to be effective for the patient, optimizing dosage of drug and detecting adverse effects that might arise through drug interactions before symptoms arise. The result of the use of calibrated profile data sets is to provide more efficient and cost effective health care management.

The novel approach described above for evaluating a biological condition of a subject may be applied to an *ex vivo* or *in vitro* assay for measuring the effect of an agent on a biological condition as illustrated in Figures 2-4. A sample from the patient may be measured directly *ex vivo* or tested *ex vivo* against an agent to predict an effect in the patient. This provides a quick and effective way to determine which drug chosen from within a single class of drugs that all may be used to treat a particular condition, may be most effective for a given subject. Alternatively, an agent may be tested on an indicator cell line that can provide a quantitative measure of therapeutic performance in a class of individuals.

Figure 2 illustrates how calibrated profile data sets may assist in screening a library of candidate compounds to discover candidate drugs. Starting with, for example, 500 candidate drugs, these can be tested in indicator cells or *ex vivo* body fluid or tissues against signature panels for *in vitro* toxicology or metabolic indicators. The figure illustrates the large number of compounds that entered in late stages in the development process only to ultimately be rejected due to adverse biological interactions. It is expected that early adoption of the use of calibrated profile data sets will more readily identify likely successful candidates and thereby reduce the expense and untoward effects of animal and human experimentation for compounds that could have been predicted to fail.

Figure 3 describes multiple screens in which a compound might be administered to an experimental animal such as a mouse or to an indicator cell line. The *in vivo* or *ex vivo* or indicator cell sample might further be treated with a stimulus. The result of both the compound and the stimulus could then be detected using to signature profiles for toxicity or for mechanism to compare the

effect of no drug +/- stimulus or +/-drug and no stimulus. Both *in vitro* (left panel) and *in vivo* (right panel) studies can be used to evaluate the effect of a compound (drug, nutraceutical, environmental stimuli, etc.). The right hand panel also illustrates the specific embodiment of an "*in vitro* clinical trial", that is, treatment of cells obtained from a subject and treated with a compound (with or without a stimulus) *in vitro* (or *ex vivo*) in order to predict the outcome of similar treatment of the subject *in vivo* (see Fig. 15 for a specific example). The output from both panels is described as toxicity and mechanistic profiles. Either experimental course may be used to both evaluate potential toxicity, e.g., using the toxicity, or liver metabolism panels, and to determine or confirm likely mechanism of action by a critical selection of a gene panel(s) that illustrates and differentiates molecular mechanisms of action (see Figure 12 for a specific example).

Figure 4 illustrates a bioassay in which cells are removed from the subject and tested *ex vivo* with the addition of a compound and also a challenge or stimulus. The *ex vivo* effect of stimulus and then drug on whole blood taken from a human subject is shown in Figure 12 in which the stimulus is lipopolysaccharide (an inflammatory agent) while the drug is any of methotrexate, meclofenamate or methylprednisolone using a signature panel for inflammation. Methylprednisolone, a drug commonly used in the treatment of acute exacerbations of COPD as well as in the chronic management of this disease, is considered to be a potent by non-specific anti-inflammatory agent. However, as demonstrated in Figure 22, its effects on gene expression are dependent on the stimulus. While there are general qualitative similarities between the effects on gene expression across these three stimuli, there are both quantitative and qualitative differences that may be important in understanding when glucocorticoid intervention is warranted.

According to embodiments of the invention, an indicator cell population is used to measure quantitative gene expression the effect of an agent or a biological sample may influence the choice of which indicator cell line will be most informative. For example, a cloned cell line such as THP-1 or a primary cell population (peripheral mononuclear cells) may provide information that is

comparable to that obtained from a body sample directly (see Figure 15). The normal state of gene expression may range from zero or few transcripts to 10^5 or more transcripts.

Similarly, an agent may be evaluated for its effect on any population of cells, either *in vivo*, *ex vivo* or *in vitro*, by administering the agent and then determining a calibrate profile data set for those cells under the selected conditions. Examples of this approach are provided in Figures 10-16 and 18. Figure 18 further provides calibrated profile data sets for different concentrations of a single agent showing that the transcription of selected constituents vary with dose and therefore the anticipated effectiveness with respect to the biological condition.

The above description of determining a biological condition is exemplified as follows. The action of a pharmaceutical or nutraceutical is measured with respect to its anti-inflammatory properties. The measurement of the effect may be established using a panel of constituent gene loci, for example, an inflammation panel, including, Interleukin 1 alpha (IL-1α) or Tumor Necrosis Factor alpha (TNF-α). The anti-inflammatory effect may first be established by treating indicator cells or sample cells *ex vivo* with a known inflammation inducers (for example, lipopolysaccharide or other mitogens) followed by treatment with the experimental agent or condition expected to suppress or reduce the expression from the appropriate gene loci. According the baseline profile data set is the delta change in gene expression for a particular panel of constituents. The addition of a potential anti-inflammatory agent results in a second delta change that is superimposed on a first delta change. This is illustrated for example in Figure 12. Methylprednisolone has a substantial down regulation effect on IL-2 in blood cells stimulated *ex vivo* with LPS where the baseline data set is LPS stimulated cells. In this case there is a negative delta. In contrast, IL-2 appears to be upregulated in whole blood not previously exposed to LPS, where the baseline data set is unstimulated cells. (Figure 16b) This is consistent with the observation that methylprednisolone stimulated IL-2 production.

The determination of the biological condition of a subject may include measuring and storing additional data about the subject. For example, if the subject is a human or mammalian patient, additional clinical indicators may be determined from blood chemistry, urinalysis, X-ray, other chemical assays and physical or sociological findings.

Figure 7 illustrates how the accumulation of calibrated profile data sets can improve the predictive power of the database and thereby increase its value in generating information about a biological condition or agent. The figure indicates the use of the database in terms of its predictive power to, for example, predict the course of a therapeutic intervention, follow the course of an individual subject compared to a population, prediction of a likely mechanism of metabolism or molecular mechanism of action or a comprehensive database that allows comparison of a single profile to a collection of signature, calibrated precision profiles.

Preferred embodiments of how the database may be used is provided in Figure 8. Figure 8 illustrates display of a data profile set from the source database. Entries for input include a name, an Experimental Type, and whether the entry is a New Reference; Cell/Tissue/Species and whether these are new; Therapeutic agent (compound), Dose, and additional parameters and whether the therapeutic agent is new. Observations are recorded according to the identity of a Gene (New Gene) and a Protein (New Protein). The Stimulus or other Treatment, if any, and the Dose are entered. Gene (and/or Protein) Expression, Expression Value, Expression Units if appropriate and Expression Time are shown. The figure specifically illustrates the range of applicable fields of investigation from complex natural products to clinical trails in humans, linkage to traditional forms of measurement and evaluation such as literature citations, clinical indicators and traditional pharmacokinetic measurements. Expert analysis of the precision profile data contained in the database may then be used to guide product development and marketing, or used to improve the clinical decision making concerning the health of a single individual or population of individuals.

It is anticipated that one form of record might provide information about a subject or agent with respect to identity, medical history including traditional pharmaceutical/medical data, clinical indications as determined from literature data, reference to additional types of analysis in the database, etc.

Figure 9 shows an embodiment of the present invention in which profile data is evaluated using data from a database that is remotely accessed over a network. The figure illustrates that data are expected to be derived at one or more locations, compared using a central database and information obtained used to affect, for example, the course of treatment of an individual or population. The two-way nature of 1109 illustrates the iterative process whereby the database affects the course of treatment or development, and outcome or response to such intervention again becomes part of the database. In a first location, as in Figure 5, from a tissue sample procured in box 1101, there are derived multiple RNA species pursuant to box 1102, and then in box 1103, profile data are quantified to produce a profile data set that is pertinent to the tissue sample obtained in box 1101. In order to evaluate the profile data set, in box 1104 information is retrieved from database 1108, which is located in a second location. In fact the database may be in communication with a large number of locations, each of which is generating profile data that must be evaluated. The retrieval of information from the database is accomplished over a network 1109, which may include the Internet, in a manner known in the art. Once information has been obtained from the database 1108, the information is used in evaluating the quantified profile data in box 1105, with the result in box 1106 that the medical condition of the subject may be assessed. The database 1108 is in box 1107 updated over the network 1109 to reflect the profile data that have been quantified in box 1103. In this manner the database 1108 may be updated to reflect the profile data obtained over all locations, and each location has the benefit of the data obtained from all of the locations.

EXAMPLES

Example 1. (a) Use of whole blood for *ex vivo* assessment of a biological condition affected by an agent.

Human blood is obtained by venipuncture and prepared for assay by

aliquoting samples for baseline, no stimulus, and stimulus with sufficient volume for at least three time points. Typical stimuli include lipopolysaccharide (LPS), phytohemagglutinin (PHA) and heat-killed staphylococci (HKS) or carrageean and may be used individually (typically) or in combination. The aliquots of heparinized, whole blood are mixed without stimulus and held at 37°C in an atmosphere of 5% CO2 for 30 minutes. Stimulus is added at varying concentrations, mixed and held loosely capped at 37°C for 30 min. Additional test compounds may be added at this point and held for varying times depending on the expected pharmacokinetics of the test compound. At defined times, cells are collected by centrifugation, the plasma removed and RNA extracted by various standard means.

(b) Preparation of RNA for Measuring Gene Expression.

Nucleic acids, RNA and or DNA are purified from cells, tissues or fluids of the test population or indicator cell lines. RNA is preferentially obtained from the nucleic acid mix using a variety of standard procedures (or RNA Isolation Strategies, pp.55-104, in RNA Methodologies, A laboratory Guide for Isolation and Characterization, 2nd edition, 1998,Robert E. Farrell, Jr., Ed., Academic Press); in the present use using a filter-based RNA isolation system from Ambion (RNAqueous™, Phenol-free Total RNA Isolation Kit, Catalog #1912, version 9908; Austin, Texas). Specific RNAs are amplified using message specific primers or random primers. The specific primers are synthesized from data obtained from public databases (e.g., Unigene, National Center for Biotechnology Information, National Library of Medicine, Bethesda, MD), including information from genomic and cDNA libraries obtained from humans and other animals. Primers are chosen to preferentially amplify from specific RNAs obtained from the test or indicator samples, see, for example, RT PCR, Chapter 15 in RNA Methodologies, A Laboratory Guide for Isolation and Characterization, 2nd edition, 1998,Robert E. Farrell, Jr., Ed., Academic Press; or Chapter 22 pp.143-151, RNA Isolation and Characterization Protocols, Methods in Molecular Biology, Volume 86, 1998, R. Rapley and D. L. Manning Eds., Human Press, or 14 in Statistical refinement of primer design parameters, Chapter 5, pp.55-72, PCR Applications: Protocols for Functional Genomics,

M.A.Innis, D.H. Gelfand and J.J. Sninsky, Eds., 1999, Academic Press). Amplifications are carried out in either isothermic conditions or using a thermal cycler (for example, a ABI 9600 or 9700 or 7700 obtained from PE Biosystems, Foster City, CA; see Nucleic Acid Detection Methods, pp. 1-24, in Molecular Methods for Virus Detection, D.L.Wiedbrauk and D.H., Farkas, Eds., 1995, Academic Press). Amplified nucleic acids are detected using fluorescent-tagged detection primers (see, for example, Taqman™ PCR Reagent Kit, Protocol, part number 402823 revision A, 1996, PE Applied Biosystems, Foster City CA.) that are identified and synthesized from publicly known databases as described for the amplification primers. In the present case, amplified DNA is detected and quantified using the ABI Prism 7700 Sequence Detection System obtained from PE Biosystems (Foster City, CA). Amounts of specific RNAs contained in the test sample or obtained from the indicator cell lines can be related to the relative quantity of fluorescence observed (see for example, Advances in Quantitative PCR Technology: 5' Nuclease Assays, Y.S. Lie and C.J. Petropolus, Current Opinion in Biotechnology, 1998, 9:43-48, or Rapid Thermal Cycling and PCR Kinetics, pp. 211-229, chapter 14 in PCR Applications: Protocols for Functional Genomics, M.A.Innis, D.H. Gelfand and J.J. Sninsky, Eds., 1999, Academic Press.

Example 2. Different inflammatory stimuli give rise to different, baseline profile data sets so that the calibrated precision profiles for different agents in the same class of anti-inflammatory result in different signature profiles.

Figure 11 documents the usefulness of different inflammatory stimuli to give rise to different, baseline profile data sets so that the calibrated precision profile data sets for the three anti-inflammatory agents tested result in different signature profiles. The different profiles reflect the difference in the molecular targets and mechanisms of action of the three agents derived from a single class of therapeutics, anti-inflammatory agents. The figure also illustrates the extraordinary range of detection (y-axis) from less than 10 fold difference from the calibrated profile to plus or minus 10E13 increase or decrease in gene expression when compared to the calibrator. Comparison to the calibrator results in gene expression profiles that are increased, decreased, or without change from the calibrated set.

Figure 11(a) shows relative gene expression (mRNA synthesis) in heat-killed staphylococci (HKS)-stimulated cells, and the effect of three different compounds (TPCK, UT-77, and "Dex", or dexamethasone). Compound TPCK caused a 10-fold decrease in relative IFN-γ expression, and 100,000-fold decreases in IL-4 and IL-5 expression. Further, compound UT-77 caused even greater magnitude of increases in relative expression of the gene encoding IL-5, and more modest increases in IL-1 expression (more than 10-fold) and IFN-γ. Such effects can be highly significant in disease etiologies and outcomes, and have predictive value concerning the usefulness as therapeutic agents of these compounds or similar chemical entities or chemicals that act similarly. HKS cells are an *in vitro* model of Gram-positive bacterial infection.

Fig. 11(b) displays analyses of expression of the 12 genes in lipopolysaccharide-(LPS)-treated cells, an *in vitro* model of Gram-negative bacterial infection. These data include several striking contrasts to the data in Fig. 11(a). Thus treatment with the therapeutic agent Dex caused a striking decrease in expression of the IL-2 gene in LPS-treated cells, and a striking increase in IL-2 expression in HKS-treated cells. Strikingly large differences in gene expression in the differently stimulated cells can be seen for the IL-4 and the IL-5 genes. Expression of the gene for IFN, in contrast, responded similarly in cells treated by either of the stimuli and any of the therapeutic agents.

By these criteria, expression of the genes for IL-2, IL-4 and IL-5 were observed to be candidate markers or surrogate markers in cell model systems to distinguish responses of the cells to Gram-positive and Gram-negative bacterial infection.

Example 3. A single therapeutic agent for treating a particular condition can be differentiated from a second therapeutic agent that also treats the particular condition by a signature profile for a given panel of gene loci.

Figure 12 shows a calibrated profile data set for a panel having 8 constituents that are indicative of a biological condition that includes inflammation. The profiles are shown for three different anti-inflammatory agents-methotrexate, meclofenamate and methylprednisolone. The calibrated profile data sets for each agent as shown represents a signature profile for that

agent. This signature profile may serve as a device for establishing quality control for a batch of the agent. Indeed, it is envisaged that compounds or classes of compounds on the market or in development may be characterized by a signature profile. The signature profile may be represented in a graphical format, more particularly as a bar graph as provided in Figure 12. For Figure 12, an *ex vivo* sample was tested. A sample of blood was taken from the subject. Aliquots of the sample were subjected to lipopolysaccharide (LPS) *ex vivo*. After 30 minutes, the anti-inflammatory agent as indicated was added to an aliquot of the sample of blood and after about another 4 hours, the expression of the panel of genes (Il-1a, Il-2, Il-8, Il-10, Il-12p35, Il-12p40, IL-15, IFN-Gamma and TNF-a) was determined. Although the calibrated profile of methotrexate and meclofenamate were similar, the calibrated profile of methylprednisolone was substantially different. Differences may be reflective of the differences of the mechanisms or target(s) of action of this agent within the general class of anti-inflammatory compounds. The baseline is the profile data set for lipopolysaccharide absent any additional agents.

Example 4. There is relatively low variability with respect to the profile within a single individual over time when the calibrated precision profile is determined from the measurement of gene expression across many gene loci that have been appropriately induced.

Figure 13(a)(b) and (c) show a graphical representation of calibrated precision profile data sets for two different samples of whole blood. Heparinized whole blood from a single normal healthy volunteer was collected on two separate occasions of more than 2 weeks apart. Figure 13a for sample 991116 and Figure 13b,for sample 991028 reflect the biological condition of the tested cells from the single donor using a panel (i.e., the inflammation panel) of 24 members, in response to stimulation with one of three different agents. The baseline in this example is derived from untreated cells obtained from the same individual. The calibrated profiles are shown for cells exposed for 4 to 6 hours to lipopolysaccharide (LPS), heat-killed Stapylococci (HKS), and phytohemagglutinin (PHA). Figure 13c shows a direct comparison of LPS-stimulated blood sample 99116 with respect to blood sample 991028, i.e., 991028

is used as the calibrator or baseline profile data set. The messenger RNA levels measured on 10/28/99 were used to compare the levels of messenger RNA measured on 11/16/99. A perfect identity of RNA levels would be represented by a flat line at unity. These data clearly show that for baseline gene expression, there can be as much as an 8 fold difference (c-jun) in messenger RNA levels. However, for most of the genes measured, the levels of messenger RNA measured on one day are within 2-3 fold of those measured on a different day. 13(d) is similar to 13(c) except that the cells were not stimulated with LPS.

The figure documents the relatively low variability with respect to the profile within a single individual over time when the calibrated precision profile is determined from the measurement of gene expression across many gene loci that have been appropriately induced. The figure illustrates (1) the class-specific effects (generally inflammatory as determined by the effect on pro-inflammatory gene loci, e.g., TNF-alpha, IL-1 alpha and IL-1 beta), (2) the agent-specific effects quantitative differences between each of the agents at the same gene loci (e.g., IL-2) and (3) reproducible and therefore predictable effects on the subject population, TK (Figure 13c)

Example 5. Similarities and differences in the effect of a single agent on cell populations differing in their biological condition.

Ex-vivo gene expression analysis can be performed by obtaining the blood of a subject for example by drawing the blood into a vacutainer tube with sodium heparin as an anticoagulant. An anti-inflammatory such as 3-methyl-prednisolone at a final concentration of 10 micromolar was added to blood in a polypropylene tube, incubated for 30 minutes at 37C. in 5% CO_2 . After 30 minutes a stimuli such as LPS at 10 ng/mL or heat killed staphlococcus (HKS) at 1:100 dilution was added to the drug treated whole blood. Incubation continued at 37C. in 5% CO_2 for 6 hours unless otherwise indicated. Erythrocytes were lysed in RBC lysis solution (Ambion) and remaining cells were lysed according to the Ambion RNAqueous-Blood module (catalog # 1913). RNA was eluted in Ambion elution solution. RNA was DNAsed treated with 1 unit of DNAse I (Ambion #2222) in 1X DNAse buffer at 37C. for 30 minutes. First strand synthesis was performed using the Perkin-Elmer TaqMan Reverse Transcriptase

kit with MultiScribe reverse transcriptase (catalog # N808-0234). Quality check of RT reactions were performed with Taqman PCR chemistry using the 18S rRNA pre-developed assay reagents (PDAR) from PE Biosystems (part #4310893E). PCR assay was performed on 6 to 24 genes in four replicates on the PE Biosystems 7700. PCR assays were performed according to specifications outlined with the PDAR product. Relative quantitation of the gene of interest was calibrated against 18S rRNA expression as described in PE product User Bulletin 2 (1997) and elaborated in Hirayama, et al (Blood 92, 1998:46-52) using 18S instead of GAPDH.

Relative quantitation of the mRNA was measured by the difference in threshold cycles between 18S and the gene of interest. This delta C_T was then compared to the normalizing condition, either subject before treatment, or stimuli without drug in an *ex-vivo* assay to measure "fold induction" represented in the bar graphs. (Figure 14) For example in the above graph, IFN-levels are 1/50 less on day 3 than before treatment.

Example 6. *In vivo* and *Ex vivo* samples provide comparable signature profiles.

Figure 15 shows the calibrated profile data set for two subjects (Subject 1 and Subject 2) who have been treated over a three day period with a standard dose of the corticosteroids, dexamethasone. Blood from each subjects was obtained 72 hours later and a quantitative measure of the amount of RNA corresponding to the panel constituents was determined. Although, the calibrated profile data set for each subject was similar for most gene loci, some notable differences were also detected, for example for Il-2, Il-10, Il-6 and GM-CSF. A calibrated profile data set is also shown for comparison for an *ex vivo* sample of blood from sample 1 prior to treatment with corticosteroid where the *ex vivo* sample is subjected to an equivalent amount of corticosteroid *in vitro* as calculated to be the plasma level in the subject. The similarity in the calibrated profile data set for *ex vivo* samples when compared to *in vivo* samples provides support for an *in vitro* assay that will predict the *in vivo* action of the compound. We have observed a similar comparable effect between *in vivo* and *ex vivo* samples infected with an infectious agent, more particularly bacterial or viral agents. We have concluded therefore that the *ex vivo* samples provide an

effective method of determining the effect of a single compound or multiple compounds on a patient, where the multiple compounds may be either used in combination, in parallel or sequentially to optimize the selection of an agent for a biological condition for the subject.

Example 7. Demonstration of reproducibility of an *in vitro* response with an approved anti-inflammatory on 5 different donor subjects.

Comparison and analysis of the Figures 18a through 18e demonstrates the consistency of effect of the stimulus and *in vitro* treatment with an approved anti-inflammatory on 5 different donors (each figure representing a unique donor). The use of a known and tested stimulus results in a highly reproducible gene response *in vitro* that may be correlated with a predictable *in vivo* response. Figures 18a-18e provide the results of analysis of 5 donors from which a blood sample has been taken. The blood samples were exposed to a therapeutic agent at various concentrations ranging from 0.1μM to 5μM, more particularly 0.1μM, 0.3μM, 1μM, 3μM and 5μM, for a 4 hour period. Different concentrations of the drug resulted in a calibrated profile data set for an inflammation panel at each concentration that was qualitatively different from the next Figure 18a corresponds to donor 1, Figure 18b corresponds to donor 2, Figure 18c corresponds to donor 3, Figure 18d corresponds to donor 4 and Figure 18e corresponds to donor 5. Each individual varied from the other and also provided a variable profile for a different concentration. This set of figures illustrates the high level of information obtainable by calibrated profile data sets.

Example 8. A calibrated profile data set may provide a signature profile for a complex mixture of compounds.

Figure 21 illustrates the effect of three different anti-inflammatory herbs on a panel of constituents including constituents of an inflammatory panel (TNF-a, Il-1b, ICAM, Il-8, Il-10, Il-12p40, ICE, cox-2, cox-1 and mmp-3) a cell growth and differentiation panel (c-fos, c-jun and STAT3) , a toxicity panel (SOD-1, TACE, GR, HSP70, GST, c-fos, c-jun, INOS) and a liver metabolism panel (INOS, cyp-a and u-pa). The cells assayed in Figure 21 are aliquots of blood from a subject that are exposed *ex vivo* to lipopolysaccharide and to Echinacea (SPM9910214) Arnica (SPM9910076) and Siberian Ginseng

(SPM9910074), each of the nutraceuticals being applied to the blood sample at the same concentration of 200ug/ml. The baseline is cell sample with lipopolysaccharide in the absence of a nutraceutical. Each nutraceutical (formed from a complex mixture) has a characteristic signature profile just as did the single compound pharmaceutical anti-inflammatory agents. The signature profile may be provided in a graphic form that can be use to identify a herbal while providing information concerning its properties and its efficacy for a single subject or for an average population of subjects.

Example 9. A quality control assay for Echinacea brands using calibrated profile data sets.

Figure 24 shows a graphic representation of the calibrated profile data sets for four different commercial brands of Echinacea Brands using an Inflammation Panel. As expected, SPM007 and SPM003 gave the signature, calibrated profiles similar to authentic Echinacea samples SPM010 and SPM 016, although labeled and sold as Echinacea when tested using the system described in Figure 14, resulted in signature calibrated profiles that were substantially similar to the profile obtained with lipopolysaccharide alone. Echinacea samples SPM010 and SPM016 were found to have elevated, highly biologically active levels of endotoxin while the LPS levels in SP007 and SP003 were undetectable. A stored signature profile for active echinacea obtained from a panel designed to test efficacy and mode of action, e.g., the inflammation panel, permits evaluation of new batches of Echinacea, differentiation of existing or new brands of Echinacea, guide the isolation and development of new compounds with different or similar activities from a complex compound like Echinacea or may be used in the development of quality assurance in the production, analysis and sale of new or previously marketed compounds. In the example cited, two of the brands of Echinacea SP010 and SP016.result in calibrated profiles that are characteristic of authentic Echinacea.

Example 10. Comparison of three herbal preparations using an indicator cell line.

Figures 25 (a)–(c) provide calibrated profile data sets for three herbal preparations with respect to an indicator cell line (THP-1) rather than a blood

sample from a subject. In Figure 25(a), the baseline is the profile data set for THP-1 cells absent the herbal while the histograms represent the calibrated profile data sets for the same herbal from three different manufacturing sources of the same herb at 250ug/ml. Gene expression results are shown on a log scale. Similar to the observation in Figure 14, these demonstrate that similarly labeled compounds obtained from different sources have demonstrable and quantifiable differences in calibrated profiles using a specific panel, eg. the inflammation panel designed to obtain information about the expression of gene products related to inflammation and infection. This suggests that the compounds likely have different efficacies when used for specific purposes.

Figure 25(b) provides a comparison of the calibrated profile of a single herb at three concentrations using the indicator cell line of THP-1. The baseline profile data set is untreated THP-1 cells. Analysis of the data suggests a concentration-dependent response in the indicator cell lines which, although demonstrated here, may be indicative of a similar response in subjects.

Figure 25(c) provides a comparison of four commercial Echinacea brands used at the same concentration and tested against a panel of constituents using a THP-1 cell line as an indicator cell population. Differential expression, as revealed by differences in the calibrated profiles, allows direct comparisons of complex compounds to be made. For example, analysis of the differences in the calibrated profiles could be used to guide compound isolation and development, product differentiation in the marketplace, or used by the consumer or health professional to guide the individualized choice of a single compound from a class of similar compounds that may be suited for a particular biological condition.

Although various embodiments of the invention have been disclosed, it should be apparent to those skilled in the art that various changes and modifications can be made which will achieve some of the advantages of the invention without departing from the true scope of the invention. These and other obvious modifications are intended to be covered by the appended claims.

What is claimed is:

1. A method, for evaluating a biological condition of a subject, comprising:

a. obtaining from the subject a sample having at least one of RNAs and proteins;

b. deriving from the sample a first profile data set, the first profile data set including a plurality of members, each member being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of the biological condition; and

c. producing a calibrated profile data set for the panel, wherein each member of the calibrated profile data set is a function of a corresponding member of the first profile data set and a corresponding member of a baseline profile data set for the panel, the calibrated profile data set providing a measure of the biological condition of the subject.

2. A method, for evaluating a biological condition of a subject, comprising:

a. obtaining from the subject a first sample having at least one of fluid, cells and active agents;

b. applying the first sample or a portion thereof to a defined population of indicator cells;

c. obtaining from the indicator cells a second sample containing at least one of RNAs or proteins;

d. deriving from the second sample a first profile data set, the first profile data set including a plurality of members, each member being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of the biological condition; and

e. producing a calibrated profile data set for the panel, wherein each member of the calibrated profile data set is a function of a

corresponding member of the first profile data set and a corresponding member of a baseline profile data set for the panel, the calibrated profile data set providing a measure of the biological condition of the subject.

3. A method, for evaluating a biological condition affected by an agent, the method comprising:

a. obtaining, from a target population of cells to which the agent has been administered, a sample having at least one of RNAs and proteins;

b. deriving from the sample a first profile data set, the first profile data set including a plurality of members, each member being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of the biological condition; and

c. producing a calibrated profile data set for the panel, wherein each member of the calibrated profile data set is a function of a corresponding member of the first profile data set and a corresponding member of a baseline profile data set for the panel, the calibrated profile data set providing a measure of the biological condition as affected by the agent.

4. A method according to any of claims 1 through 2, wherein the baseline profile data set is derived from one or more other samples from the same subject taken under conditions different from those of the sample.

5. A method according to claim 4, wherein the conditions are selected from the group consisting of (i) the time at which a given sample is taken, (ii) the site from which a given sample is taken, (iii) the physiological condition of the subject when a given sample is taken.

6. A method according to claim 4, wherein the one or more other samples are taken over an interval of time that is at least twelve months between an initial sample and the sample.

7. A method according to claim 4, wherein the one or more other samples are taken over an interval of time that is at least one month between an initial sample and the sample.

8. A method according to any of claims 1 through 3, wherein the sample is derived from blood and the baseline profile data set is derived from tissue or body fluid of the subject other than blood.

9. A method according to claim 4, wherein the baseline profile data set is derived from one or more other samples from the same subject, taken when the subject is in a physiological condition different from that in which the subject was at the time the sample was taken, with respect to at least one of age, diet, medication, and environmental exposure.

10. A method according to claim 3, wherein the baseline profile data set is derived from one or more other samples from the same population taken under conditions different from those of the sample.

11. A method according to claim 10, wherein the conditions are selected from the group consisting of (i) the time at which a given sample is taken and (ii) the physiological condition of the population when a given sample is taken.

12. A method according to claim 10, wherein the one or more other samples are taken over an interval of time that is at least twelve months between an initial sample and the sample.

13. A method according to claim 10, wherein the one or more other samples are taken over an interval of time that is at least one month between an initial sample and the sample.

14. A method according to claim 10, wherein the sample is derived from blood and the baseline profile data set is derived from tissue or body fluid of the subject other than blood.

15. A method according to claim 10, wherein the baseline profile data set is derived from one or more other samples of cell populations associated with a common subject, the populations taken when the subject is in a physiological condition different from that in which the subject was at the time the sample was taken, with respect to at least one of age, diet, medication, and environmental exposure.

16. A method according to any of claims 1 and 2, wherein the baseline profile data set is derived from one or more other samples from one or more different subjects.

17. A method according to claim 16, wherein the one or more different subjects have in common with the subject at least one of age group, gender, ethnicity, geographic location, diet, medical disorder, clinical indicator, medication, physical activity, body mass, and environmental exposure.

18. A method according to claim 3, wherein the baseline profile data set is derived from one or more other samples from one or more cell populations associated with different subjects.

19. A method according to claim 18, wherein the one or more different subjects have in common with the subject at least one of age group, gender, ethnicity, geographic location, diet, medical disorder, clinical indicator, medication, physical activity, body mass, and environmental exposure.

20. A method according to any of claims 1 through 3, further comprising: interpreting the calibrated profile data set in the context of at least one other clinical indicator.

21. A method according to claim 20, wherein the indicator is selected from the group consisting of blood chemistry, urinalysis, X-ray, other chemical assays, and physical findings.

22. A method according to any of claims 1 through 3, wherein the biological condition is a complex disease process, involving multiple genes, the disease being of a type involving at least one of inflammation, auto-immune disease, degenerative disease, allergy, vascular disease, ischemia, cancer, developmental disease, hormonal condition, aging and infectious diseases.

23. A method according to claim 22, wherein the biological condition is one of arthritis, asthma, multiple sclerosis, and perimenopausal change.

24. A method according to any of claims 1 and 2, wherein the subject is a living organism.

25. A method according to claim 24, wherein the subject is a mammal.

26. A method according to claim 3, wherein the population of cells is human cells.

27. A method according to claim 3, wherein the population of cells is mammalian cells.

28. A method according to any of claims 1 through 3, wherein the sample is derived from one or more of body fluid and tissue.

29. A method according to any of claims 1 through 3, wherein the sample is derived from blood.

30. A method according to any of claims 1 through 3, wherein the

sample is derived from one of a biopsy, a needle aspirate, a lavage specimen, a scraping, and a surgical specimen.

31. A method according to any of claims 1 through 3, wherein the sample is derived from tissue or fluid of a type distinct from that with respect to which the condition is clinically manifested.

32. A method according to any of claims 1 through 3, wherein the condition is a disease and the sample is derived from tissue or fluid of a type distinct from that which is a primary target of the disease.

33. A method according to any of claims 1 through 3, wherein the function is other than a simple difference.

34. A method according to claim 33, wherein the function is a second function of the ratio of the corresponding member of first profile data set to the corresponding member of the baseline profile data set.

35. A method according to claim 34, wherein the function is a logarithmic function.

36. A method according to any of claims 1 through 3, wherein each member of the calibrated profile data set is reproducible with respect to similar samples taken from the subject under similar conditions.

37. A method according to any of claims 1 through 3, wherein each member of the calibrated profile data set is reproducible within one order of magnitude with respect to similar samples taken from the subject under similar conditions.

38. A method according to any of claims 1 through 3, wherein each member of the calibrated profile data set is reproducible within fifty percent

with respect to similar samples taken from the subject under similar conditions.

39. A method according to any of claims 1 through 3, wherein each member of the calibrated profile data set is reproducible within twenty percent with respect to similar samples taken from the subject under similar conditions.

40. A method according to claim 34, wherein each member of the calibrated profile data set is reproducible within one order of magnitude with respect to similar samples taken from the subject under similar conditions.

41. A method according to claim 34, wherein each member of the calibrated profile data set is reproducible within fifty percent with respect to similar samples taken from the subject under similar conditions.

42. A method according to claim 34, wherein each member of the calibrated profile data set is reproducible within twenty percent with respect to similar samples taken from the subject under similar conditions.

43. A method according to claim 34, wherein each member of the calibrated profile data set has biological significance if it has a value differing by more than an amount D, where D = F(1.1) – F(.9), and F is the second function.

44. A method according to any of claims 1 through 3, wherein the biological condition concerns an organ and the panel of constituents enables measurement of the condition in relation to the organ.

45. A method according to any of claims 1 and 2, wherein the biological condition concerns a system of the subject, the system selected from the group consisting of respiratory, vascular, nervous, metabolic, urinary, reproductive, structural, and immunological systems, and the panel of constituents enables measurement of the condition of the subject in relation to the system.

46. A method according to claim 3, wherein the population of cells is derived from a subject and the biological condition concerns a system of the subject, the system selected from the group consisting of respiratory, vascular, nervous, metabolic, urinary, reproductive, structural, and immunological systems, and the panel of constituents enables measurement of the condition of the subject in relation to the system.

47. A method according to claim 46 and the panel includes at least half of the constituents of the Inflammation Panel.

48. A method according to claim 46 and the panel includes at least eighty percent of the constituents of the Inflammation Panel.

49. A method according to claim 46 and the panel includes at least half of the constituents of the Cell Growth and Differentiation Panel.

50. A method according to claim 46 and the panel includes at least eighty percent of the constituents of the Cell Growth and Differentiation Panel.

51. A method according to claim 46 and the panel includes at least half of the constituents of a Toxicity Panel.

52. A method according to claim 46 and the panel includes at least eighty percent of the constituents of a Toxicity Panel.

53. A method according to any of claims 1 through 3, wherein the number of constituents in the panel is at least three but less than 100.

54. A method according to any of claims 1 through 3, wherein the number of constituents in the panel is at least four but less than 100.

55. A method according to any of claims 1 through 3, wherein the number of constituents in the panel is at least at least five but less than 100.

56. A method according to any of claims 1 through 3, wherein the number of constituents in the panel is at least is at least six.

57. A method according to claim 3, wherein the agent is selected from the group consisting of a drug, a mixture of compounds, a functional food, a nutraceutical, a therapeutic agent, an allergen, and a toxin.

58. A method according to any of claims 1 through 3, wherein deriving the first profile data set from the sample includes hybridizing the sample with a set of nucleic acid probes.

59. A method according to claim 58, wherein the probes are attached to an insoluble matrix and the sample is applied to the matrix.

60. A method according to claim 3, wherein evaluating the condition affected by the agent includes evaluating the interaction of the agent with a second agent administered to the population of cells.

61. A method according to claim 60, wherein the interaction is neutral.

62. A method according to claim 60, wherein the interaction is interference.

63. A method according to claim 60, wherein the interaction is cumulative.

64. A method according to claim 60, wherein the interaction is synergistic.

65. A method according to claim 60, wherein the agent is a pharmaceutical.

66. A method, for evaluating the effect on a biological condition by a first agent in relation to the effect by a second agent, the method comprising:

a. obtaining, from first and second target populations of cells to which the first and second agents have been respectively administered, first and second samples respectively, each sample having at least one of RNAs and proteins;

b. deriving from the first sample a first profile data set and from the second sample a second profile data set, the profile data sets each including a plurality of members, each member being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of the biological condition; and

c. producing for the panel a first calibrated profile data set and a second profile data set, wherein (i) each member of the first calibrated profile data set is a function of a corresponding member of the first profile data set and a corresponding member of a first baseline profile data set for the panel, and (ii) each member of the second calibrated profile data set is a function of a corresponding member of the second profile data set and a corresponding member of a second baseline profile data set for the panel, the calibrated profile data sets providing a measure of the effect by the first agent on the biological condition in relation to the effect by the second agent.

67. A method according to claim 66, wherein the first agent is a drug and the second agent is a complex mixture.

68. A method according to claim 66, wherein the first agent is a drug and the second agent is a nutriceutical.

69. A method according to any of claims 1 through 3, wherein

obtaining the sample and quantifying the first profile data set are performed at a first location, and producing the calibrated profile data set includes using a network to access a database stored on a digital storage medium in a second location.

70. A method according to claim 69, further comprising updating the database to reflect the first profile data set quantified from the sample.

71. A method according to claim 69, wherein using a network includes accessing a global computer network.

72. A method of conducting a clinical trial of an agent, the method comprising:

a. causing the blind administration of a selected one of a placebo and the agent to each candidate of a pool of subjects; and

b. using quantitative gene expression to monitor an effect of such administration.

73. A method according to claim 72, wherein the pool of subjects is selected using quantitative gene expression analysis on a plurality of candidates to identify those candidates likely to show a response to the agent.

74. A method according to claim 72, wherein the administration includes determining at least one of a dosage and a dosage range by using quantitative gene expression analysis.

75. A method according to claim 72, further comprising using quantitative gene expression analysis to assist in determining at least one of efficacy and toxicity of the agent.

76. A method according to any of claims 72 through 75, wherein using quantitative gene expression analysis includes using the method of at least one

of claims 1, 2, and 3.

77. A digital storage medium on which is stored a computer readable calibrated profile data set, wherein:

a. the calibrated profile data set relates to a sample having at least one of RNAs and proteins derived from a target cell population to which an agent has been administered; and

b. the calibrated profile data set includes a first plurality of members, each member being a quantitative measure of a change in an amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of a biological condition as affected by administration of the agent.

78. A digital storage medium according to claim 77, wherein: (i) each member of the calibrated profile data set is a function of a corresponding member of a post-administration data set and a corresponding member of a baseline data set; (ii) each member of the baseline data set is a quantitative measure of the amount of a distinct RNA or protein constituent in the panel under a normative condition; and (iii) each member of the post-administration data set is a quantitative measure of the amount of a distinct RNA or protein constituent in the panel under a condition following administration of the agent to the target cell population.

79. A medium according to claim 78, wherein the function is a second function of the ratio of the corresponding member of baseline data set to the corresponding member of the post-administration data set.

80. A medium according to claim 79, wherein the second function is a logarithmic function.

81. A digital storage medium according to any of claims 77-80, wherein the agent is a pharmaceutical.

82. A digital storage medium according to any of claims 77-80, wherein the agent includes a second plurality of components.

83. A digital storage medium according to any of claims 77-80, wherein the agent is a nutraceutical.

84. A digital storage medium according to any of claims 77-80, wherein the first plurality is at least three but less than 1000.

85. A digital storage medium according to any of claims 77-80, wherein the first plurality is at least four but less than 1000.

86. A digital storage medium according to any of claims 77-80, wherein the first plurality is at least five but less than 1000.

87. A digital storage medium according to any of claims 77-80, wherein the first plurality is at least six.

88. A digital storage medium on which is stored a plurality of records R_i relating to a population of subjects, each record R_i corresponding to a distinct instance P_i of a computer readable profile data set P wherein:

a. each instance P_i of the profile data set P relates to a distinct sample derived from a subject, the sample having at least one of RNAs and proteins;

b. the profile data P set includes a plurality of members M_j, each member M_j being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of a biological condition;

c. each record R_i includes, for each member M_{ij} of a corresponding distinct instance P_i of the profile data set P, a value corresponding to the value of the member M_{ij}; and

d. each record R_j also includes a reference to a characteristic of the subject relative to the record, the characteristic being at least one of age group, gender, ethnicity, geographic location, diet, medical disorder, clinical indicator, medication, physical activity, body mass, and environmental exposure.

89. A digital storage medium according to claim 88, wherein each sample is derived from a target cell population to which has been administered an agent, such target cell population being derived from a subject.

90. A digital storage medium on is stored a large number of computer readable profile data sets, wherein:

a. each profile data set relates to a sample derived from a target cell population to which has been administered an agent, the sample having at least one of RNAs and proteins;

b. each profile data set includes a plurality of members, each member being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of a biological condition; and

c. the panel is the same for all profile data sets.

91. A method, for evaluating a biological condition of a subject, based on a sample from the subject, the sample having at least one of RNAs and proteins, the method comprising:

a. deriving from the sample a first instance of a profile data set, the profile data set including a plurality of members, each member being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of the biological condition; and

b. producing a first instance of a calibrated profile data set for the panel, wherein each member of an instance of the calibrated profile data set is a function of a corresponding member of an instance of the profile data set

and a corresponding member of an instance of a baseline profile data set for the panel, the calibrated profile data set providing a measure of the biological condition of the subject; and

c. accessing a data in a condition database, the condition database having a plurality of records relating to a population of subjects, each record corresponding to a distinct instance of the calibrated profile data set; and

d. evaluating the first instance of the calibrated profile data set in relation to data in the condition database.

92. A method according to claim 91, wherein accessing the condition database includes accessing the condition database over a network.

93. A method according to claim 92, wherein the network is a global computer network.

94. A method according to claim 92, further comprising: supplementing the condition database based on data associated with the first instance of the calibrated profile data set.

95. A method according to claim 92, wherein the biological condition concerns a system of the subject, the system selected from the group consisting of respiratory, vascular, nervous, metabolic, urinary, reproductive, structural, and immunological systems and the panel of constituents enables measurement of the condition of the subject in relation to the system.

96. A method according to claim 92, wherein each record also references a characteristic of the population relative to the record, the characteristic being at least one of age group, gender, ethnicity, geographic location, diet, medical disorder, clinical indicator, medication, physical activity, body mass, and environmental exposure.

97. A method according to claim 96, wherein the characteristic includes a clinical indicator.

98. A method of displaying quantitative gene expression analysis data associated with measurement of a biological condition, the method comprising:

a. identifying a first profile data set pertinent to the gene expression analysis data, the first profile data set including a plurality of members, each member being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of the biological condition;

b. producing a calibrated profile data set for the panel, wherein each member of the calibrated profile data set is a function of a corresponding member of the first profile data set and a corresponding member of a baseline profile data set for the panel, the calibrated profile data set providing a measure of the biological condition of the subject; and

c. displaying the calibrated profile data set in a graphical format.

99. A method according to claim 98, wherein the function is a second function of the ratio of the corresponding member of first profile data set to the corresponding member of the baseline profile data set.

100. A method according to claim 97, wherein the function is a logarithmic function.

101. A method according to claim 97, wherein the graphical format is a bar graph for each member of the calibrated profile data set.

102. A descriptive record of a change in a biological condition in a population of cells, comprising:

a. a first set of numerical gene expression values for a panel of

gene loci, each value in the set corresponding to a single gene locus in a panel of gene loci, the set of values forming a profile data set for a population of cells subjected to a first biological condition;

b. a second set of numerical gene expression values for the panel of gene loci, each value in the set corresponding to a single gene locus, the set of values forming a baseline profile data set for a second population of cells subjected to a second biological condition, the second set of values optionally being an average for multiple gene expression values from multiple populations of cells for each locus in the panel; and

c. a third set of numbers corresponding to the ratio of the first set of values and the second set of values with respect to each gene locus in the panel, the third set being a calibrated profile data set; the profile data set and the calibrated profile data set being descriptive of the first biological condition with respect to the second biological condition.

103. A record according to claim 102, wherein the first population of cells and the second or more population of cells are the same population of cells.

104. A record according to claim 102, wherein the first population of cells and the second or more population of cells are different populations of cells.

105. A descriptive record, according to any of claims 102 through 104, wherein a sample is obtained from a subject, for subjecting the cells to a biological condition, the cell population being an indicator cell population.

106. A gene expression profile data set, according to any of claims 102 through 104, wherein the population of cells is in a subject or derived from a subject.

107. A method for diagnosing a biological condition of a subject, comprising :

obtaining a sample from a subject; subjecting a population of cells to the sample and determining the presence of a first biological condition with respect to a second biological condition according to any of claims 1 through 3.

108. A method according to claim 107, further comprising: selecting the subject for a clinical trial according to the biological condition of the subject, so as to determine predictively whether the subject will respond to a test compound if the compound has a predetermined biological activity.

109. A method according to claim 108, wherein the test compound is a pharmaceutical agent.

110. A method according to claim 108 where the test compound is a nutraceutical agent.

111. A method for diagnosing a susceptibility for a biological condition in a subject, comprising:

a. obtaining a sample from the subject;

b. creating a descriptive record, according to any of claims 102 through 106, wherein the set of baseline values is an average of second values contained in a library of descriptive records for the second biological condition; the library containing a plurality of records grouped according to a predetermined biological condition; and

c. diagnosing the susceptibility of the subject.

112. A method for monitoring the progress of a biological condition, comprising:

a. creating a plurality of descriptive records, according to any of claims 102 through 106, wherein each set of first values is determined at preselected time intervals with respect to each of the other gene expression profiles;

b. comparing each calibrated profile data set with a library of calibrated profile data sets, the plurality of calibrated profile data sets being grouped according to a predetermined biological condition; and

c. determining the progress of the biological condition.

113. A method of establishing the biological activity of a composition, comprising:

a. selecting a population of cells;

b. subjecting the cells to the composition; and

c. determining the record according to any of claims 102 through 106 using a standardized baseline profile data set for the biological condition.

114. A method according to claim 113, wherein the composition is a nutraceutical.

115. A method according to claim 113, wherein the composition is a pharmaceutical.

116. A method according to claim 113, wherein the composition is an infectious agent.

117. A method according to claim 113, wherein the composition is a complex mixture.

118. A method according to claim 113, wherein establishing the biological activity of the composition further includes providing a mechanism of action for the composition.

119. A method according to claim 113, wherein establishing the biological activity of the composition further includes providing a mechanism for metabolism for the composition.

120. A method according to claim 113, wherein the composition further comprises a first compound and a second compound and the biological activity results from any of synergism, interference or neutral interaction between the first and second compound.

121. A method according to claim 113, wherein the compound further comprises a plurality of compounds such that the biological activity results from any of synergism, interference or neutral interaction between the compounds.

122. A method according to claim 113, wherein the biological activity of the compound is a toxic effect on the subject.

123. A method of determining which therapeutic agent from a choice of a plurality of therapeutic agents to administer to a subject so as to change a biological condition in a subject from a first biological condition to a second biological condition; comprising:

a. subjecting a sample from the subject to each of a plurality of therapeutic agents;

b. determining a descriptive record for each of the samples according to any of claims 102 through 106;

c. comparing each of the calibrated profile data sets to a library of calibrated profile data sets; wherein the library of calibrated profile data sets being grouped according to a predetermined biological condition; and

d. determining which of the therapeutic agents is capable of changing the first biological condition in the subject to the second biological condition in the subject.

124. A method according to claim 122, wherein the first biological condition is a consequence of the adverse effects of any of an infectious agent, a biological warfare agent or an environmental agent and the second biological condition is a reversal of these adverse effects.

125. A method according to claim 122, wherein the library of descriptive records comprise a medical history for a single subject or condition.

126. A method according to claim 122, wherein the library of descriptive records comprise medical information about a plurality of subjects or conditions.

127. A method according to claim 122, wherein the library of signature profile data sets consist of signature profile data sets from a plurality of subjects.

128. A method for characterizing the biological effectiveness of a single batch of a composition produced by a manufacturing process, comprising: providing a fingerprint according to the method of claim 112; and labeling the batch of the composition by placing the fingerprint on each container in the batch.

129. A method for accessing biological information on a digital storage medium according to claim 88, comprising: making the information available to a user.

130. A method according to claim 129, wherein the method further comprises making the information available to the user on any of a network, World Wide Web, email, internet access site or hard copy.

131. A method according to claim 128, wherein the method further comprises accessing the information for loading to a second access site.

132. A method according to claim 131, wherein the process for loading includes downloading the information.

133. A method according to claim 129, wherein access to the information is controlled.

134. A method according to claim 133, wherein the process of control includes the use of a password.

135. A method according to claim 129, wherein the user can annotate the available information, the annotation becoming part of the biological information.

136. A method according to claim 129, wherein the user can add one or more records to the data set, the one or more records becoming part of the biological information.

137. A method for consumer evaluation of a product, wherein the consumer evaluation is dependent on a signature profile according to claim 102, comprising: identifying the product using the signature profile.

138. A method according to claim 137, wherein the product is promoted according to the signature profile.

139. A computer program product for evaluating a biological condition of a subject or for evaluating a biological condition resulting from the use of an agent, including a computer usable medium having computer readable program code thereon, the computer program code; comprising:

a. a program code for classifying a sample from the subject or the agent for an identifiable record;

b. a program code for deriving a first data set, the first profile data set including a plurality of members, each member being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of the biological condition; the profile data set being stored in the

record; and

c. a program code for optionally producing a calibrated profile data set for the panel, for storage in the record, each member of the calibrated profile data set being a function of a corresponding member of the first profile data set and a corresponding member of a baseline profile data set for the panel, the calibrated profile data set providing a measure of the biological condition of the subject.

140. A computer system for evaluating a biological condition of a subject or for evaluating a biological condition resulting from the use of an agent, the computer system, comprising:

a. a classification module for classifying a sample from the subject or the agent in an identifiable record

b. a derivative module for deriving a first data set, the first profile data set including a plurality of members, each member being a quantitative measure of the amount of a distinct RNA or protein constituent in a panel of constituents selected so that measurement of the constituents enables measurement of the biological condition; and

c. a production module for producing a calibrated profile data set for the panel, wherein each member of the calibrated profile data set is a function of a corresponding member of the first profile data set and a corresponding member of a baseline profile data set for the panel, the calibrated profile data set providing a measure of the biological condition of the subject.

141. A method for analyzing a patient for a biological condition at a remote site, comprising:

a. providing a kit for measuring a profile data base for evaluating a biological condition, the kit including reagents for quantitative analysis of RNA or protein for a panel of gene loci;

b. accessing a centralized database containing baseline profile data sets corresponding to the panel;

c. determining the calibrated profile data set for the patient;

and

d. analyzing the biological condition of the patient.

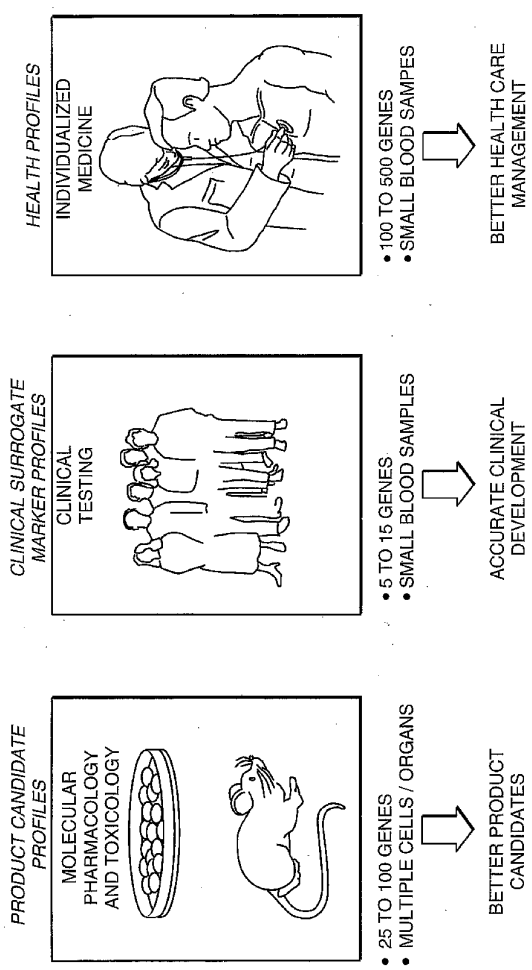

HEALTH PROFILES
INDIVIDUALIZED MEDICINE
• 100 TO 500 GENES
• SMALL BLOOD SAMPES
BETTER HEALTH CARE MANAGEMENT
CLINICAL SURROGATE MARKER PROFILES
CLINICAL TESTING
• 5 TO 15 GENES
• SMALL BLOOD SAMPLES
ACCURATE CLINICAL DEVELOPMENT
PRODUCT CANDIDATE PROFILES
MOLECULAR PHARMACOLOGY AND TOXICOLOGY
• 25 TO 100 GENES
• MULTIPLE CELLS / ORGANS
BETTER PRODUCT CANDIDATES

FIG. 1

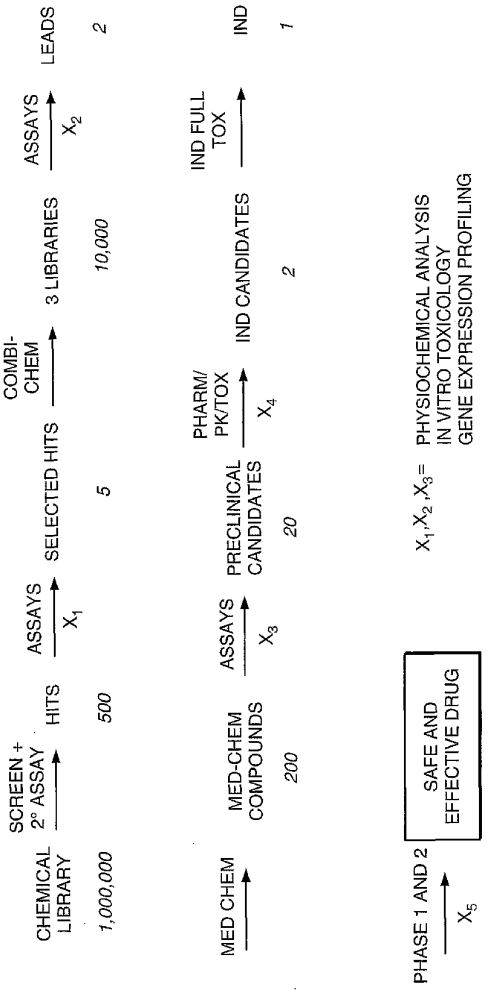

CHEMICAL LIBRARY
1,000,000
SCREEN + 2° ASSAY
HITS
500
ASSAYS
X1
SELECTED HITS
5
COMBI-CHEM
3 LIBRARIES
10,000
ASSAYS
X2
LEADS
2
MED CHEM
MED-CHEM COMPOUNDS
200
ASSAYS
X3
PRECLINICAL CANDIDATES
20
PHARM/ PK/TOX
X4
IND CANDIDATES
2
IND FULL TOX
IND
1
PHASE 1 AND 2
X5
SAFE AND EFFECTIVE DRUG
X1, X2, X3 = PHYSIOCHEMICAL ANALYSIS IN VITRO TOXICOLOGY GENE EXPRESSION PROFILING
X4 = IN VIVO GENE PROFILING
X5 = SURROGATE MARKER

FIG. 2

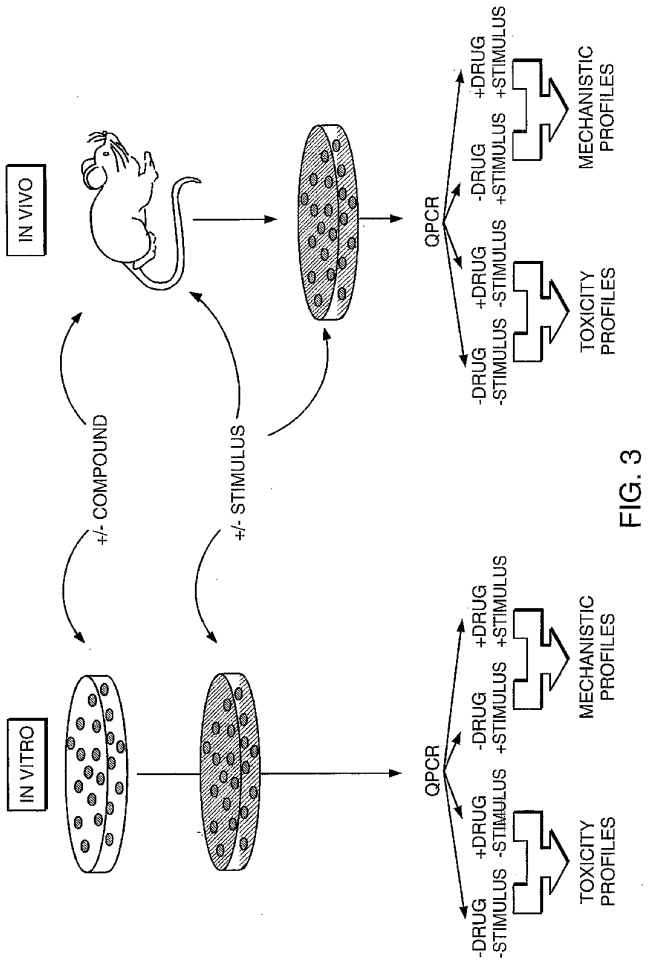

IN VIVO
IN VITRO
+/- COMPOUND
+/- STIMULUS
QPCR
-DRUG -STIMULUS
+DRUG -STIMULUS
-DRUG +STIMULUS
+DRUG +STIMULUS
TOXICITY PROFILES
MECHANISTIC PROFILES

FIG. 3

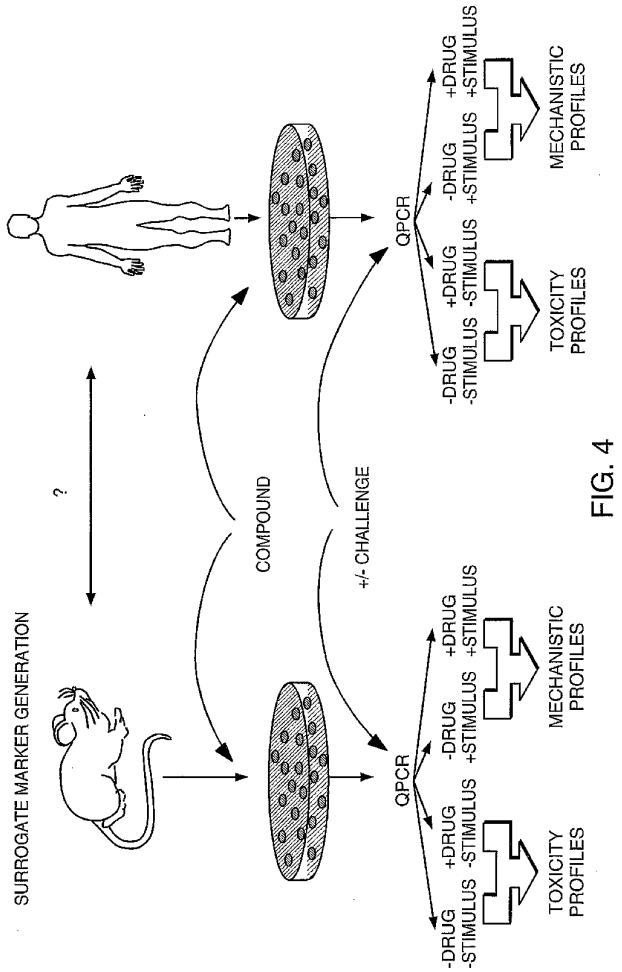

SURROGATE MARKER GENERATION
?
COMPOUND
+/- CHALLENGE
QPCR
QPCR
-DRUG -STIMULUS
+DRUG -STIMULUS
-DRUG +STIMULUS
+DRUG +STIMULUS
TOXICITY PROFILES
MECHANISTIC PROFILES
-DRUG -STIMULUS
+DRUG -STIMULUS
-DRUG +STIMULUS
+DRUG +STIMULUS
TOXICITY PROFILES
MECHANISTIC PROFILES

FIG. 4

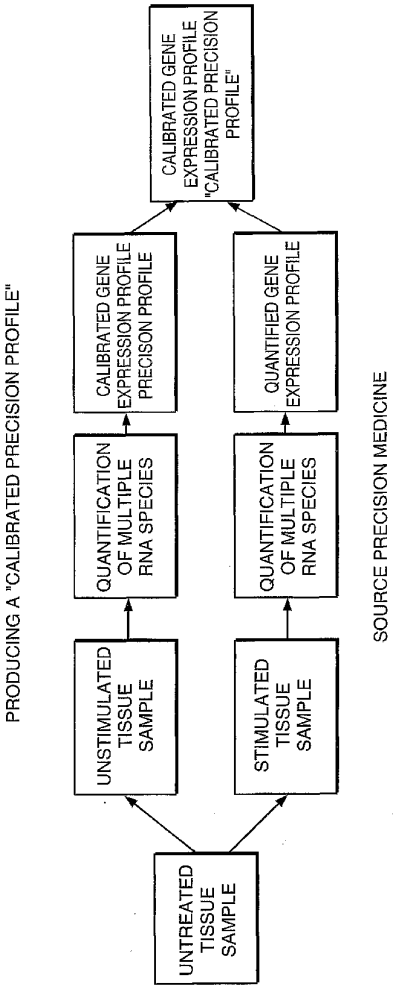

PRODUCING A "CALIBRATED PRECISION PROFILE"
UNTREATED TISSUE SAMPLE
UNSTIMULATED TISSUE SAMPLE
STIMULATED TISSUE SAMPLE
QUANTIFICATION OF MULTIPLE RNA SPECIES
QUANTIFICATION OF MULTIPLE RNA SPECIES
CALIBRATED GENE EXPRESSION PROFILE PRECISON PROFILE
QUANTIFIED GENE EXPRESSION PROFILE
CALIBRATED GENE EXPRESSION PROFILE "CALIBRATED PRECISION PROFILE"
SOURCE PRECISION MEDICINE

FIG. 5

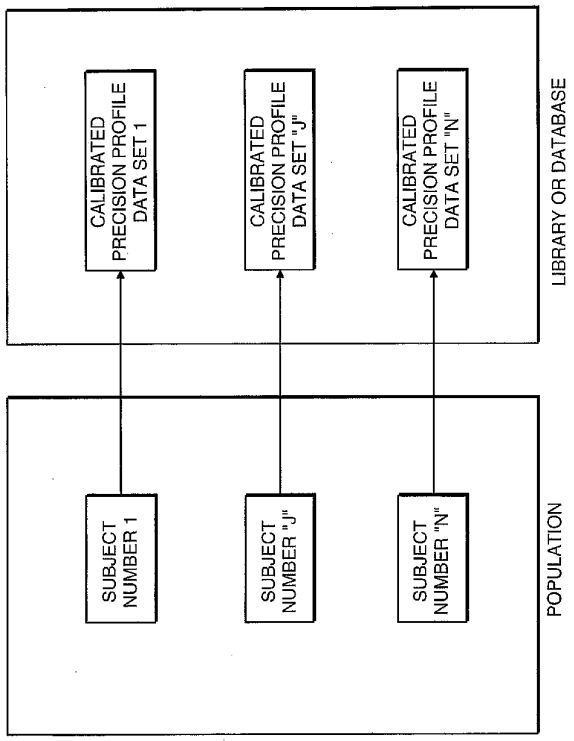

CALIBRATED PRECISION PROFILE DATA SET 1
CALIBRATED PRECISION PROFILE DATA SET "J"
CALIBRATED PRECISION PROFILE DATA SET "N"
LIBRARY OR DATABASE
SUBJECT NUMBER 1
SUBJECT NUMBER "J"
SUBJECT NUMBER "N"
POPULATION

FIG. 6

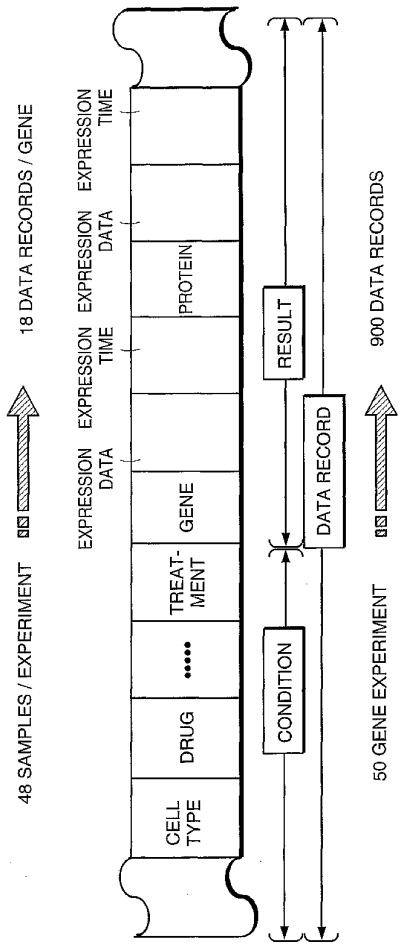

48 SAMPLES / EXPERIMENT
18 DATA RECORDS / GENE
CELL TYPE
DRUG
.....
TREAT-MENT
GENE
EXPRESSION DATA
EXPRESSION TIME
EXPRESSION DATA
EXPRESSION TIME
PROTEIN
EXPRESSION DATA
EXPRESSION TIME
CONDITION
RESULT
DATA RECORD
50 GENE EXPERIMENT
900 DATA RECORDS
EACH NEW RECORD IMPROVES THE PREDICTIVE POWER OF THE DATABASE AND INCREASES ITS VALUE

FIG. 7

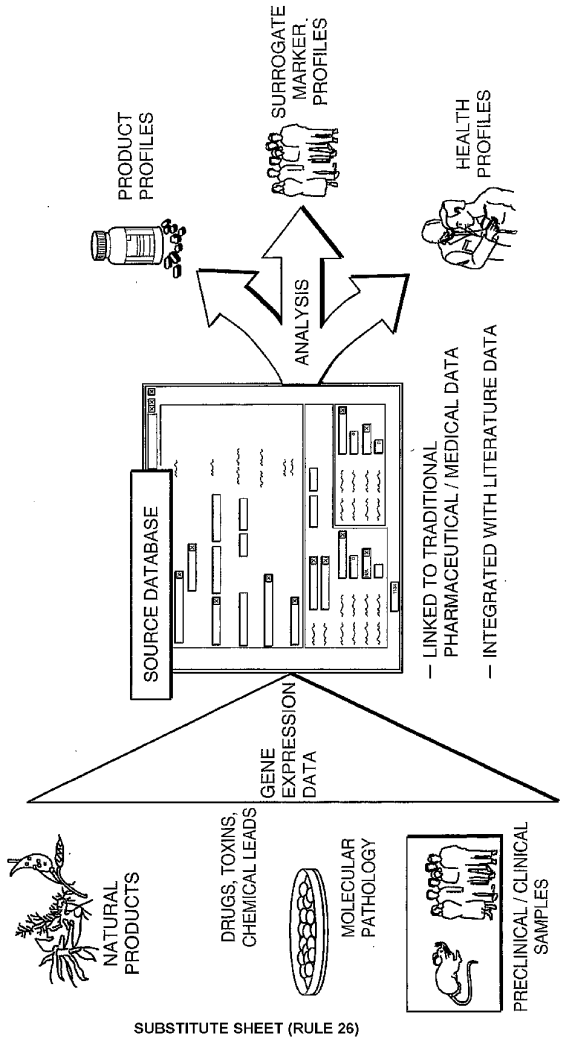

PRODUCT PROFILES
SURROGATE MARKER. PROFILES
HEALTH PROFILES
ANALYSIS
SOURCE DATABASE
– LINKED TO TRADITIONAL PHARMACEUTICAL / MEDICAL DATA
– INTEGRATED WITH LITERATURE DATA
GENE EXPRESSION DATA
NATURAL PRODUCTS
DRUGS, TOXINS, CHEMICAL LEADS
MOLECULAR PATHOLOGY
PRECLINICAL / CLINICAL SAMPLES

FIG. 8

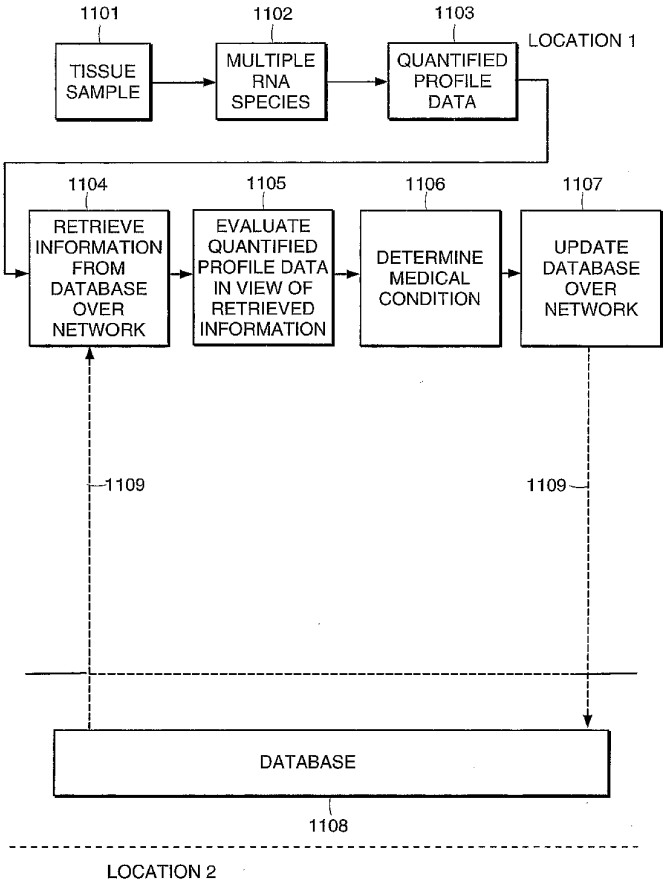

1101
TISSUE SAMPLE
1102
MULTIPLE RNA SPECIES
1103
QUANTIFIED PROFILE DATA
LOCATION 1
1104
RETRIEVE INFORMATION FROM DATABASE OVER NETWORK
1105
EVALUATE QUANTIFIED PROFILE DATA IN VIEW OF RETRIEVED INFORMATION
1106
DETERMINE MEDICAL CONDITION
1107
UPDATE DATABASE OVER NETWORK
1109
1109
DATABASE
1108
LOCATION 2

FIG. 9

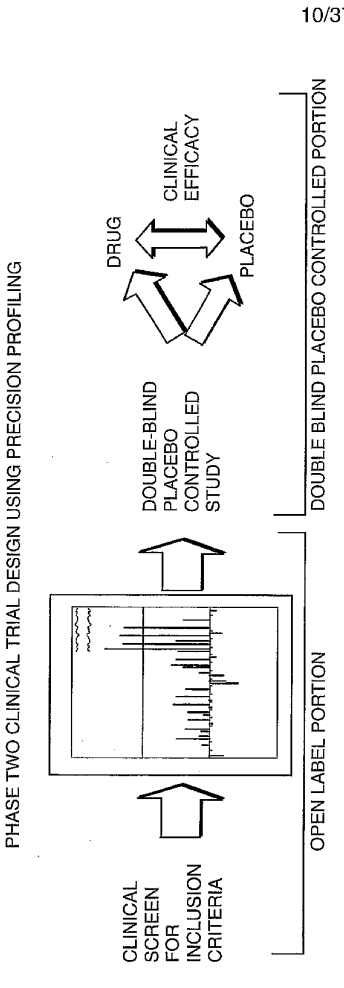


FIG. 10a

FIG. 10b

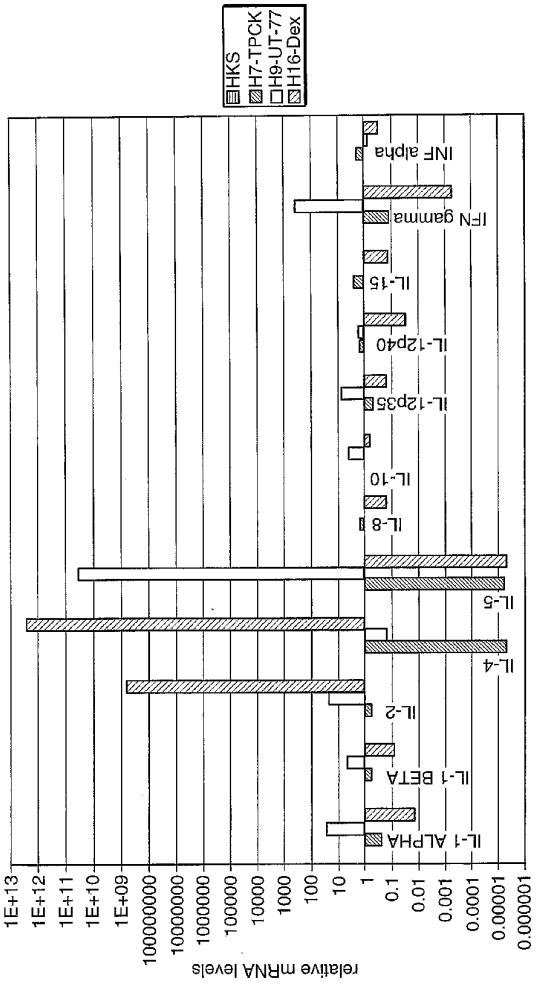

HKS
H7-TPCK
H9-UT-77
H16-Dex
relative mRNA levels
1E+13
1E+12
1E+11
1E+10
1E+09
100000000
10000000
1000000
100000
10000
1000
100
10
1
0.1
0.01
0.001
0.0001
0.00001
0.000001
IL-1 ALPHA
IL-1 BETA
IL-2
IL-4
IL-5
IL-8
IL-10
IL-12p35
IL-12p40
IL-15
IFN gamma
INF alpha

FIG. 11a

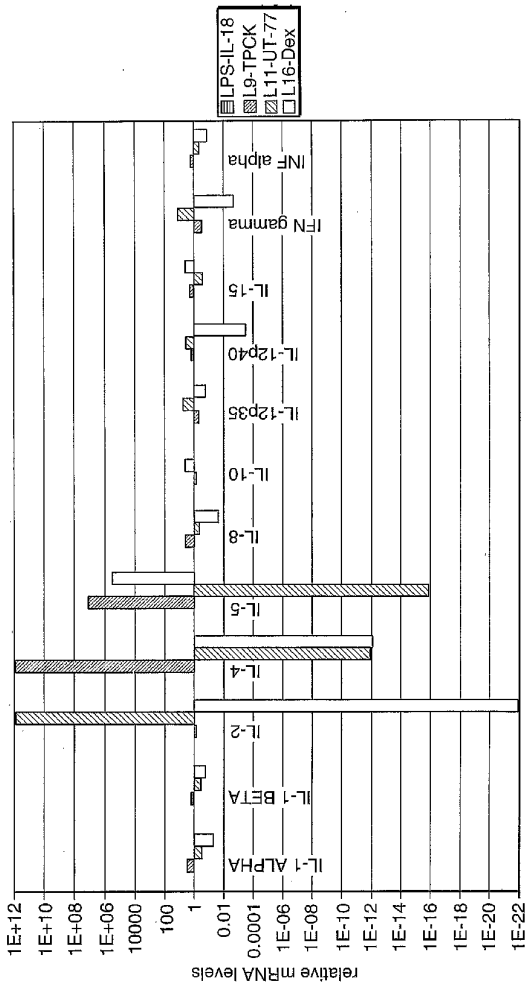

LPS-IL-18
L9-TPCK
L11-UT-77
L16-Dex
INF alpha
IFN gamma
IL-15
IL-12p40
IL-12p35
IL-10
IL-8
IL-5
IL-4
IL-2
IL-1 BETA
IL-1 ALPHA
1E+12
1E+10
1E+08
1E+06
10000
100
1
0.01
0.0001
1E-06
1E-08
1E-10
1E-12
1E-14
1E-16
1E-18
1E-20
1E-22
relative mRNA levels

FIG. 11b

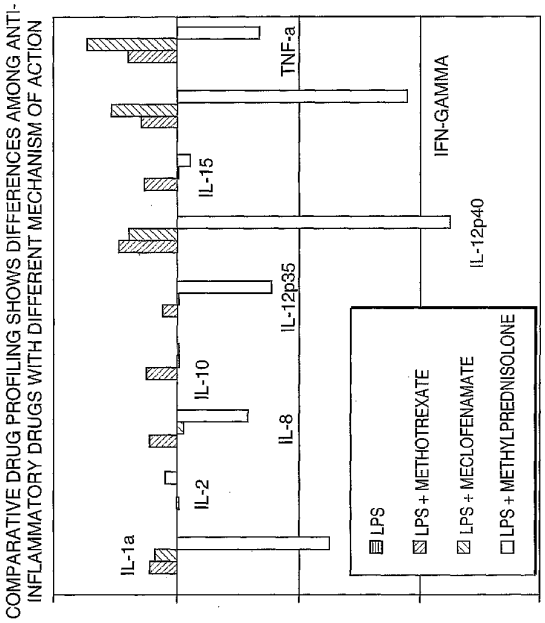

COMPARATIVE DRUG PROFILING SHOWS DIFFERENCES AMONG ANTI-INFLAMMATORY DRUGS WITH DIFFERENT MECHANISM OF ACTION
IL-1a
IL-2
IL-10
IL-8
IL-12p35
IL-15
TNF-a
IFN-GAMMA
IL-12p40
LPS
LPS + METHOTREXATE
LPS + MECLOFENAMATE
LPS + METHYLPREDNISOLONE

FIG. 12a

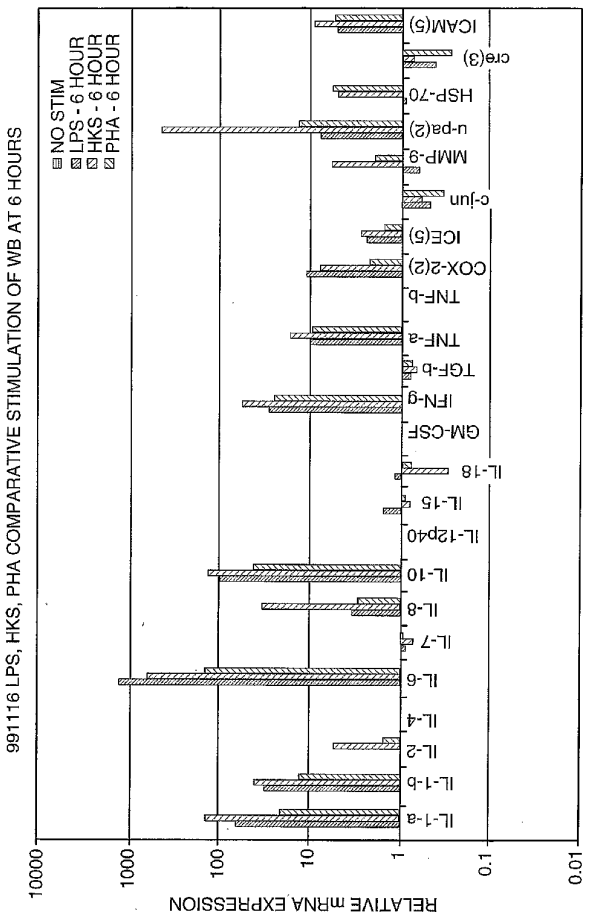

991116 LPS, HKS, PHA COMPARATIVE STIMULATION OF WB AT 6 HOURS
NO STIM
LPS - 6 HOUR
HKS - 6 HOUR
PHA - 6 HOUR
RELATIVE mRNA EXPRESSION
10000
1000
100
10
1
0.1
0.01
IL-1-a
IL-1-b
IL-2
IL-4
IL-6
IL-7
IL-8
IL-10
IL-12p40
IL-15
IL-18
GM-CSF
IFN-g
TGF-b
TNF-a
TNF-b
COX-2(2)
ICE(5)
c-jun
MMP-9
u-pa(2)
HSP-70
cre(3)
ICAM(5)

FIG. 13a

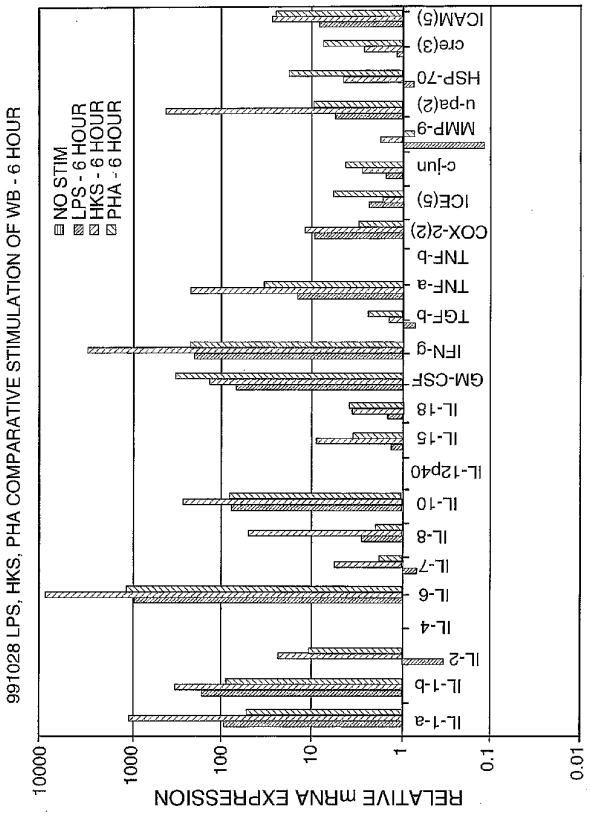

991028 LPS, HKS, PHA COMPARATIVE STIMULATION OF WB - 6 HOUR
NO STIM
LPS - 6 HOUR
HKS - 6 HOUR
PHA - 6 HOUR
RELATIVE mRNA EXPRESSION
10000
1000
100
10
1
0.1
0.01
IL-1-a
IL-1-b
IL-2
IL-4
IL-6
IL-7
IL-8
IL-10
IL-12p40
IL-15
IL-18
GM-CSF
IFN-g
TGF-b
TNF-a
TNF-b
COX-2(2)
ICE(5)
c-jun
MMP-9
u-pa(2)
HSP-70
cre(3)
ICAM(5)

FIG. 13b

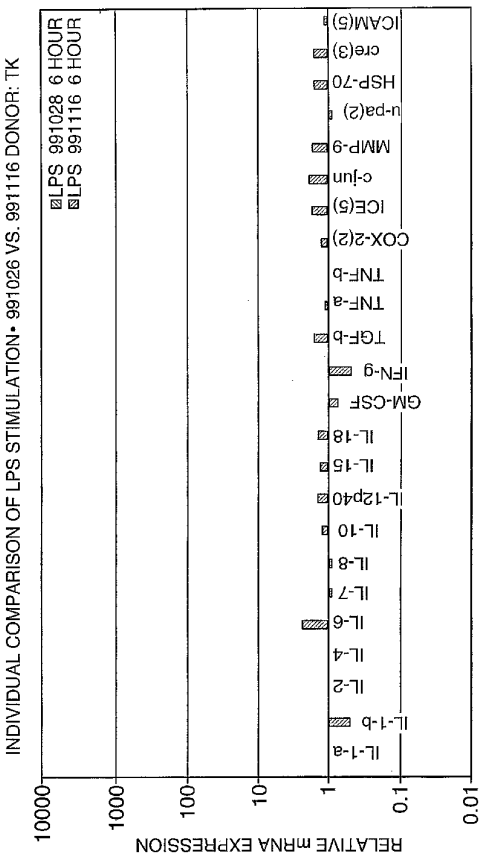

INDIVIDUAL COMPARISON OF LPS STIMULATION• 991026 VS. 991116 DONOR: TK
LPS 991028 6 HOUR
LPS 991116 6 HOUR
RELATIVE mRNA EXPRESSION
10000
1000
100
10
1
0.1
0.01
IL-1-a
IL-1-b
IL-2
IL-4
IL-6
IL-7
IL-8
IL-10
IL-12p40
IL-15
IL-18
GM-CSF
IFN-g
TGF-b
TNF-a
TNF-b
COX-2(2)
ICE(5)
c-jun
MMP-9
u-pa(2)
HSP-70
cre(3)
ICAM(5)

FIG. 13c

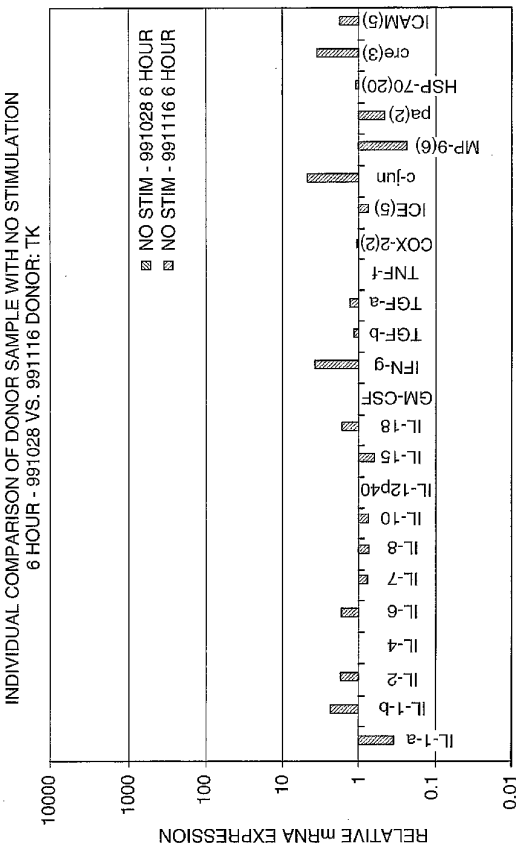

INDIVIDUAL COMPARISON OF DONOR SAMPLE WITH NO STIMULATION
6 HOUR - 991028 VS. 991116 DONOR: TK
NO STIM - 991028 6 HOUR
NO STIM - 991116 6 HOUR
RELATIVE mRNA EXPRESSION
10000
1000
100
10
1
0.1
0.01
IL-1-a
IL-1-b
IL-2
IL-4
IL-6
IL-7
IL-8
IL-10
IL-12p40
IL-15
IL-18
GM-CSF
IFN-g
TGF-b
TGF-a
TNF-f
COX-2(2)
ICE(5)
c-jun
MP-9(6)
pa(2)
HSP-70(20)
cre(3)
ICAM(5)

FIG. 13d

FIG. 14

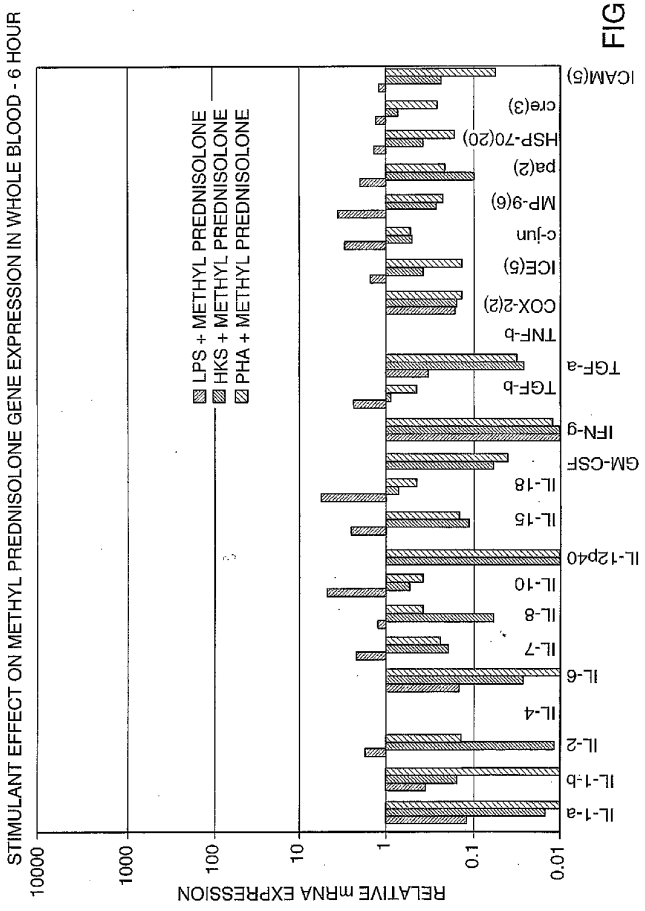
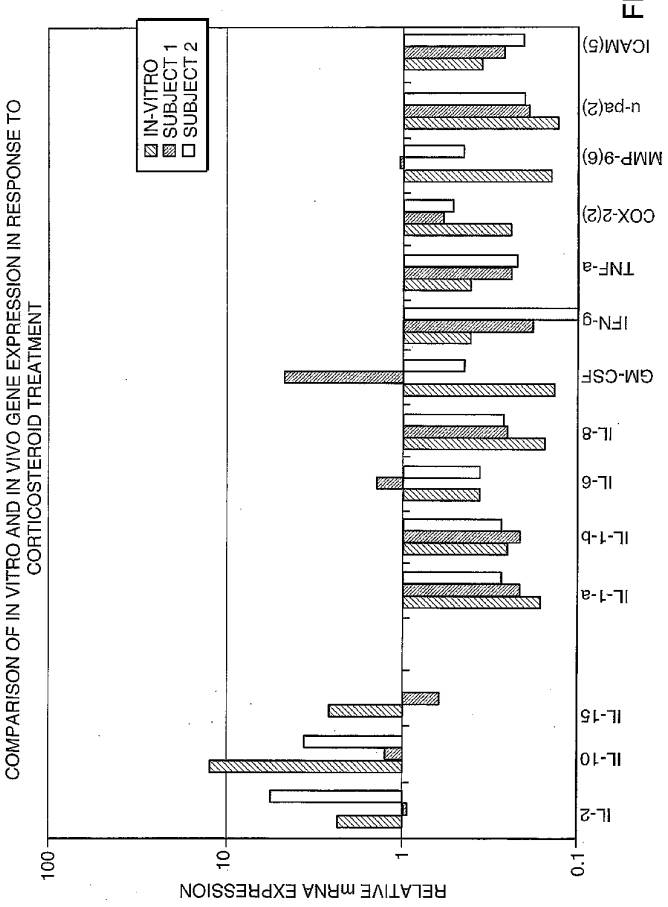

STIMULANT EFFECT ON METHYL PREDNISOLONE GENE EXPRESSION IN WHOLE BLOOD - 6 HOUR
LPS + METHYL PREDNISOLONE
HKS + METHYL PREDNISOLONE
PHA + METHYL PREDNISOLONE
RELATIVE mRNA EXPRESSION
10000
1000
100
10
1
0.1
0.01
IL-1-a
IL-1-b
IL-2
IL-4
IL-6
IL-7
IL-8
IL-10
IL-12p40
IL-15
IL-18
GM-CSF
IFN-g
TGF-b
TGF-a
TNF-b
COX-2(2)
ICE(5)
c-jun
MP-9(6)
pa(2)
HSP-70(20)
cre(3)
ICAM(5)

FIG. 15


COMPARISON OF IN VITRO AND IN VIVO GENE EXPRESSION IN RESPONSE TO CORTICOSTEROID TREATMENT
IN-VITRO
SUBJECT 1
SUBJECT 2
RELATIVE mRNA EXPRESSION
100
10
1
0.1
IL-2
IL-10
IL-15
IL-1-a
IL-1-b
IL-6
IL-8
GM-CSF
IFN-g
TNF-a
COX-2(2)
MMP-9(6)
u-pa(2)
ICAM(5)

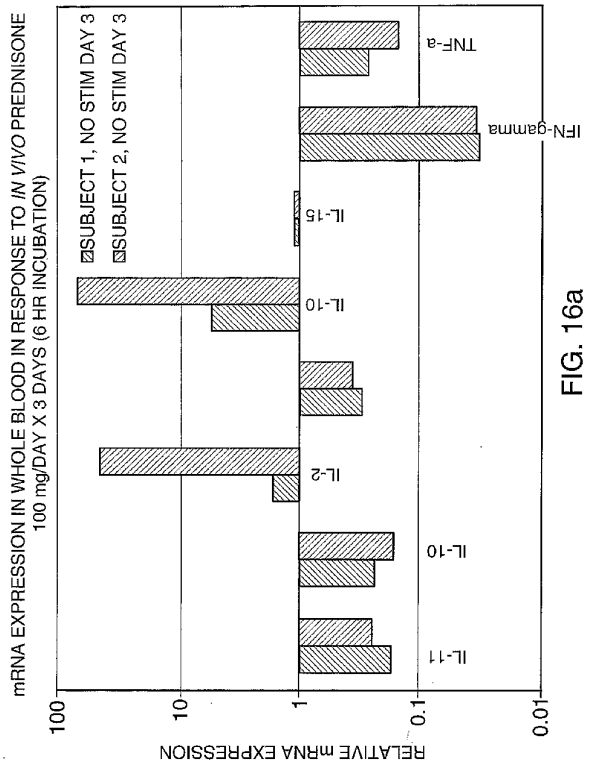

mRNA EXPRESSION IN WHOLE BLOOD IN RESPONSE TO IN VIVO PREDNISONE
100 mg/DAY X 3 DAYS (6 HR INCUBATION)
SUBJECT 1, NO STIM DAY 3
SUBJECT 2, NO STIM DAY 3
RELATIVE mRNA EXPRESSION
100
10
1
0.1
0.01
TNF-a
IFN-gamma
IL-15
IL-10
IL-2
IL-10
IL-11

FIG. 16a

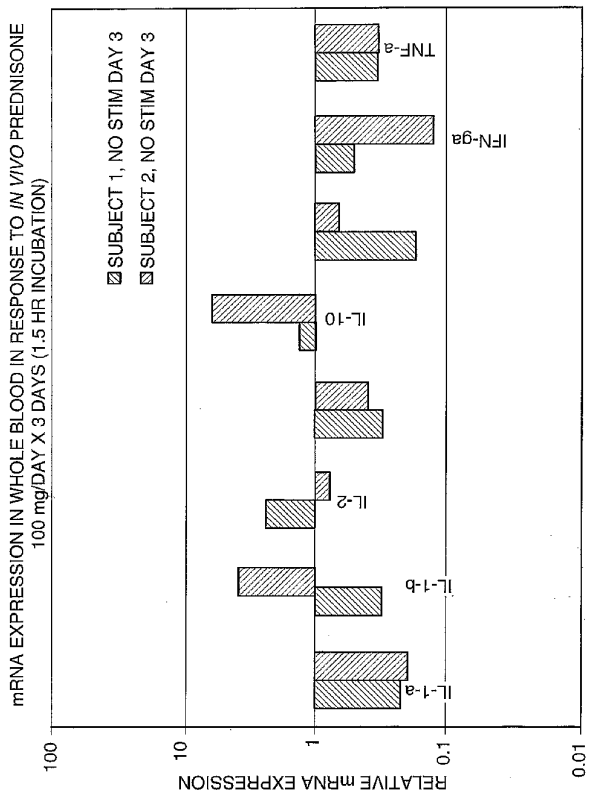

mRNA EXPRESSION IN WHOLE BLOOD IN RESPONSE TO IN VIVO PREDNISONE
100 mg/DAY X 3 DAYS (1.5 HR INCUBATION)
SUBJECT 1, NO STIM DAY 3
SUBJECT 2, NO STIM DAY 3
RELATIVE mRNA EXPRESSION
100
10
1
0.1
0.01
TNF-a
IFN-ga
IL-10
IL-2
IL-1b
IL-1a

FIG. 16b

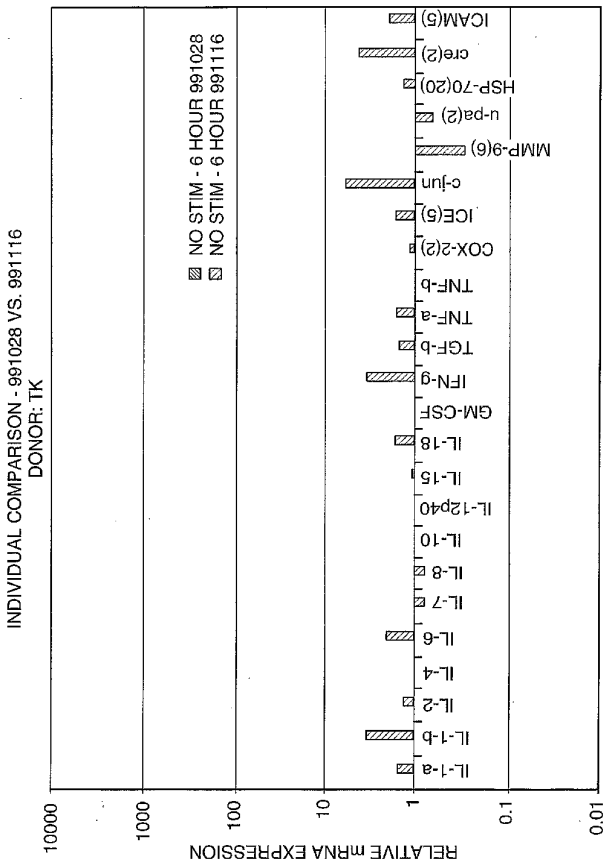

INDIVIDUAL COMPARISON - 991028 VS. 991116
DONOR: TK
NO STIM - 6 HOUR 991028
NO STIM - 6 HOUR 991116
RELATIVE mRNA EXPRESSION
10000
1000
100
10
1
0.1
0.01
IL-1-a
IL-1-b
IL-2
IL-4
IL-6
IL-7
IL-8
IL-10
IL-12p40
IL-15
IL-18
GM-CSF
IFN-g
TGF-b
TNF-a
TNF-b
COX-2(2)
ICE(5)
c-jun
MMP-9(6)
u-pa(2)
HSP-70(20)
cre(2)
ICAM(5)

FIG. 17

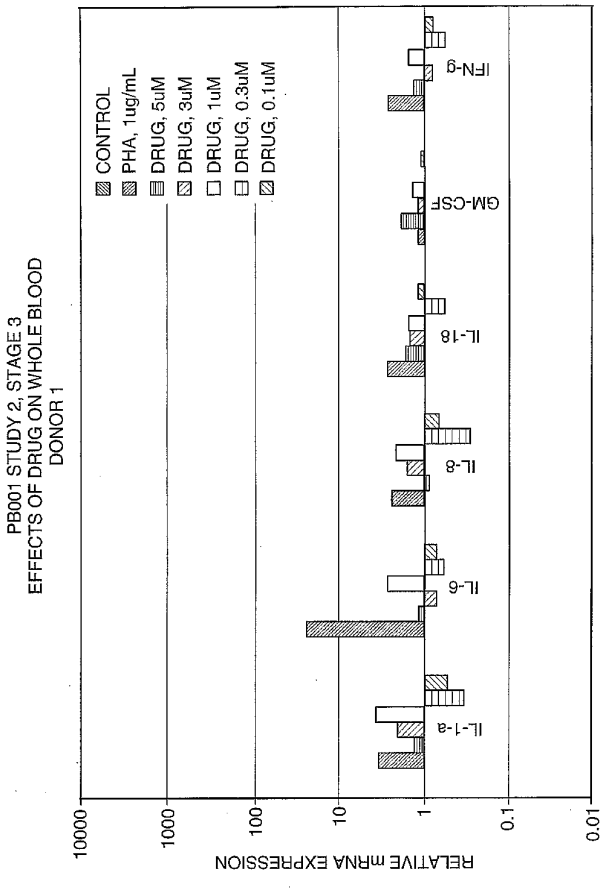

PB001 STUDY 2, STAGE 3
EFFECTS OF DRUG ON WHOLE BLOOD
DONOR 1
CONTROL
PHA, 1ug/mL
DRUG, 5uM
DRUG, 3uM
DRUG, 1uM
DRUG, 0.3uM
DRUG, 0.1uM
IL-1-a
IL-6
IL-8
IL-18
GM-CSF
IFN-g
RELATIVE mRNA EXPRESSION
10000
1000
100
10
1
0.1
0.01

FIG. 18a

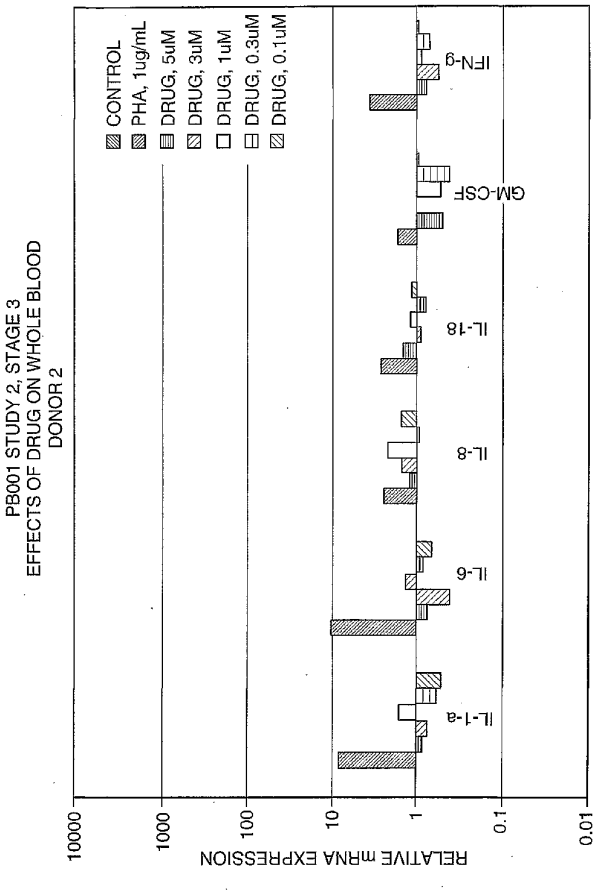

PB001 STUDY 2, STAGE 3
EFFECTS OF DRUG ON WHOLE BLOOD
DONOR 2
CONTROL
PHA, 1ug/mL
DRUG, 5uM
DRUG, 3uM
DRUG, 1uM
DRUG, 0.3uM
DRUG, 0.1uM
IFN-g
GM-CSF
IL-18
IL-8
IL-6
IL-1-a
RELATIVE mRNA EXPRESSION
10000
1000
100
10
1
0.1
0.01

FIG. 18b

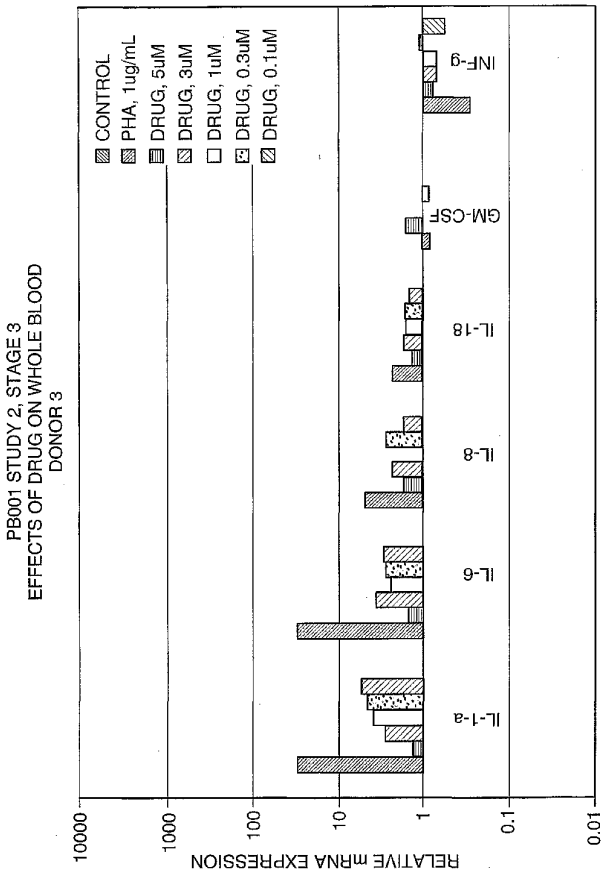

PB001 STUDY 2, STAGE 3
EFFECTS OF DRUG ON WHOLE BLOOD
DONOR 3
CONTROL
PHA, 1ug/mL
DRUG, 5uM
DRUG, 3uM
DRUG, 1uM
DRUG, 0.3uM
DRUG, 0.1uM
IL-1-a
IL-6
IL-8
IL-18
GM-CSF
INF-g
RELATIVE mRNA EXPRESSION
10000
1000
100
10
1
0.1
0.01

FIG. 18c

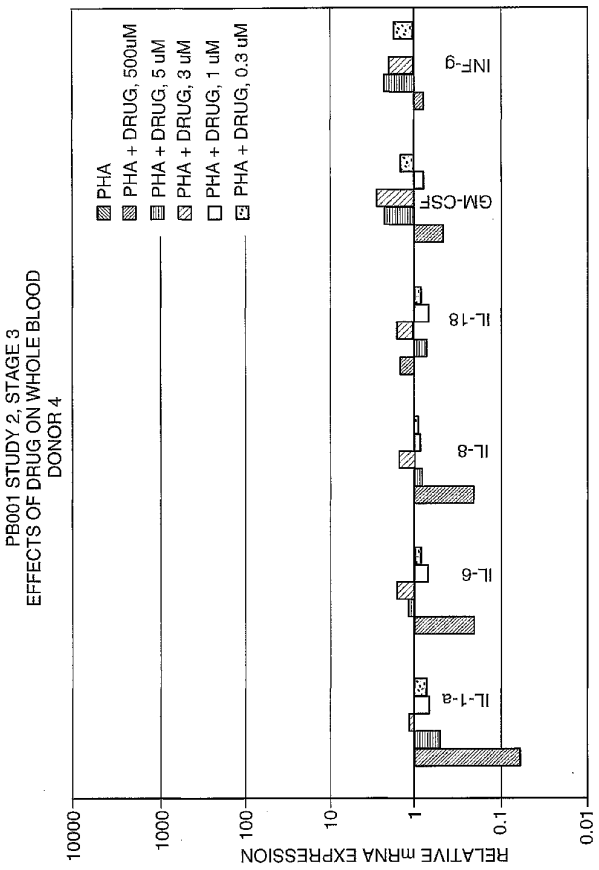

PB001 STUDY 2, STAGE 3
EFFECTS OF DRUG ON WHOLE BLOOD
DONOR 4
PHA
PHA + DRUG, 500uM
PHA + DRUG, 5 uM
PHA + DRUG, 3 uM
PHA + DRUG, 1 uM
PHA + DRUG, 0.3 uM
IL-1-a
IL-6
IL-8
IL-18
GM-CSF
INF-g
RELATIVE mRNA EXPRESSION
10000
1000
100
10
1
0.1
0.01

FIG. 18d

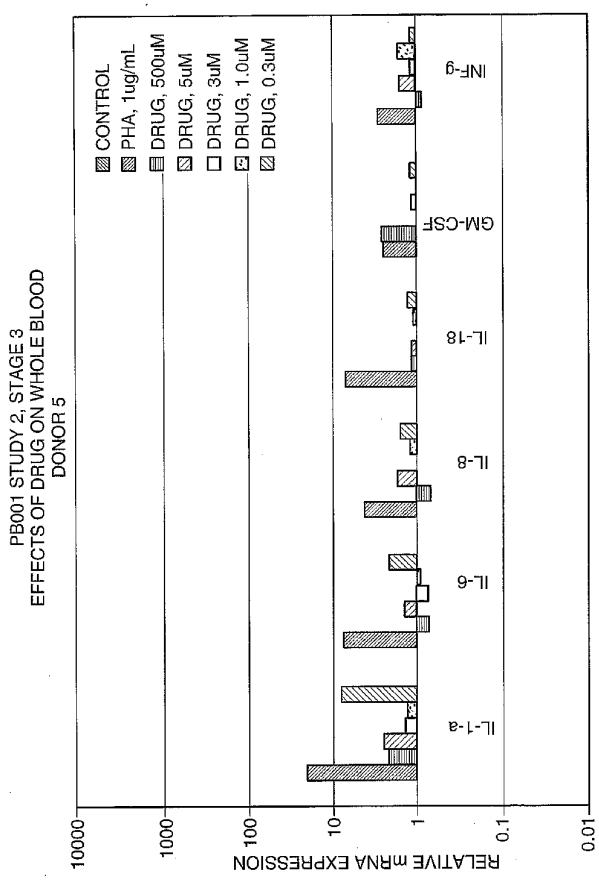

PB001 STUDY 2, STAGE 3
EFFECTS OF DRUG ON WHOLE BLOOD
DONOR 5
CONTROL
PHA, 1ug/mL
DRUG, 500uM
DRUG, 5uM
DRUG, 3uM
DRUG, 1.0uM
DRUG, 0.3uM
RELATIVE mRNA EXPRESSION
10000
1000
100
10
1
0.1
0.01
IL-1-a
IL-6
IL-8
IL-18
GM-CSF
INF-g

FIG. 18e

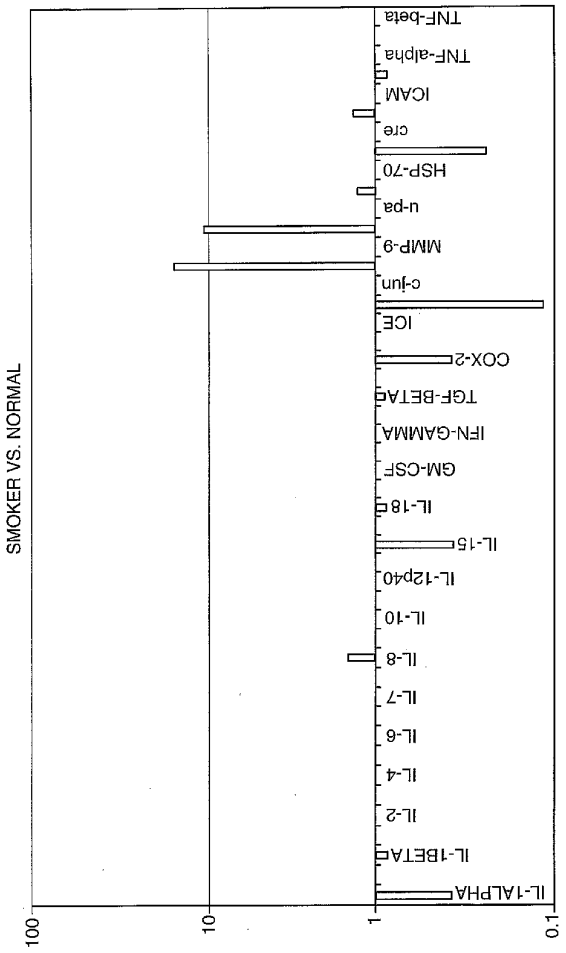

SMOKER VS. NORMAL
100
10
1
0.1
IL-1ALPHA
IL-1BETA
IL-2
IL-4
IL-6
IL-7
IL-8
IL-10
IL-12p40
IL-15
IL-18
GM-CSF
IFN-GAMMA
TGF-BETA
COX-2
ICE
c-jun
MMP-9
u-pa
HSP-70
cre
ICAM
TNF-alpha
TNF-beta

FIG. 19a

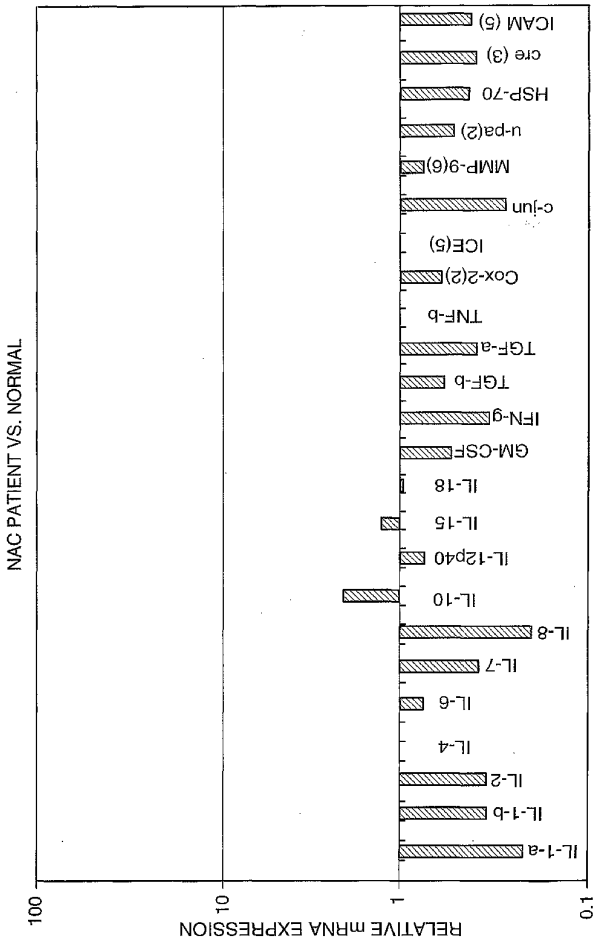

NAC PATIENT VS. NORMAL
RELATIVE mRNA EXPRESSION
100
10
1
0.1
IL-1-a
IL-1-b
IL-2
IL-4
IL-6
IL-7
IL-8
IL-10
IL-12p40
IL-15
IL-18
GM-CSF
IFN-g
TGF-b
TGF-a
TNF-b
Cox-2(2)
ICE(5)
c-jun
MMP-9(6)
u-pa(2)
HSP-70
cre (3)
ICAM (5)

FIG. 19b

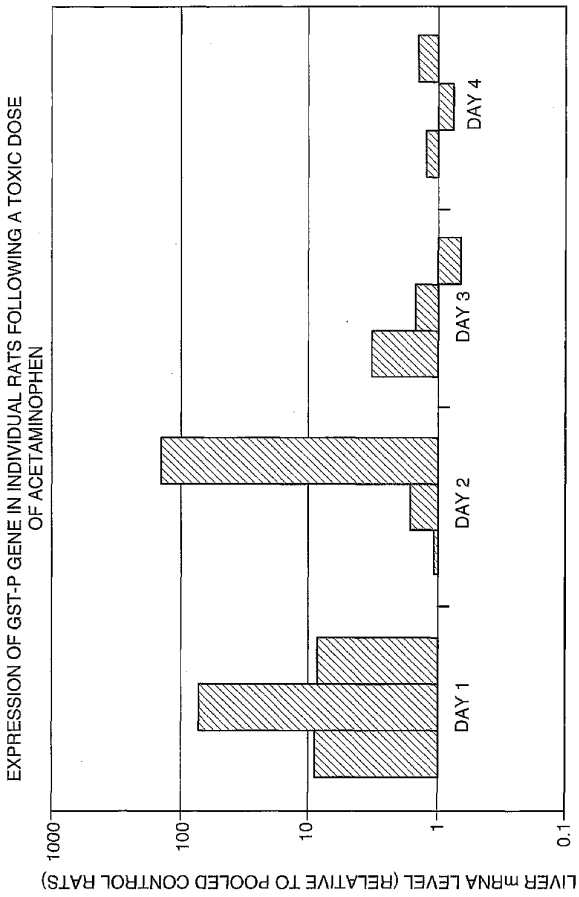

EXPRESSION OF GST-P GENE IN INDIVIDUAL RATS FOLLOWING A TOXIC DOSE OF ACETAMINOPHEN
LIVER mRNA LEVEL (RELATIVE TO POOLED CONTROL RATS)
1000
100
10
1
0.1
DAY 1
DAY 2
DAY 3
DAY 4

FIG. 20

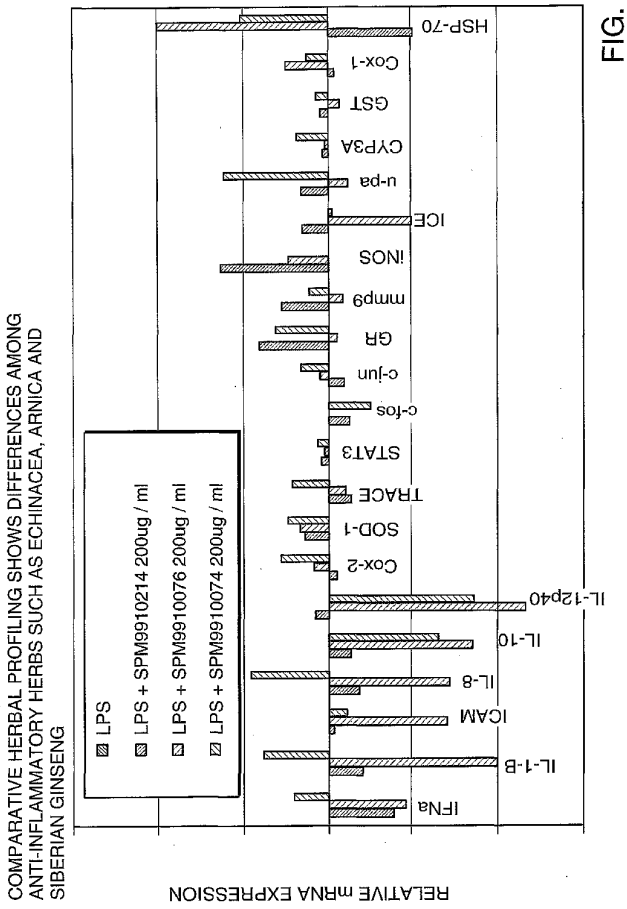

COMPARATIVE HERBAL PROFILING SHOWS DIFFERENCES AMONG ANTI-INFLAMMATORY HERBS SUCH AS ECHINACEA, ARNICA AND SIBERIAN GINSENG
LPS
LPS + SPM9910214 200ug / ml
LPS + SPM9910076 200ug / ml
LPS + SPM9910074 200ug / ml
IFNa
IL-1-B
ICAM
IL-8
IL-10
IL-12p40
Cox-2
SOD-1
TRACE
STAT3
c-fos
c-jun
GR
mmp9
iNOS
ICE
u-pa
CYP3A
GST
Cox-1
HSP-70
RELATIVE mRNA EXPRESSION

FIG. 21

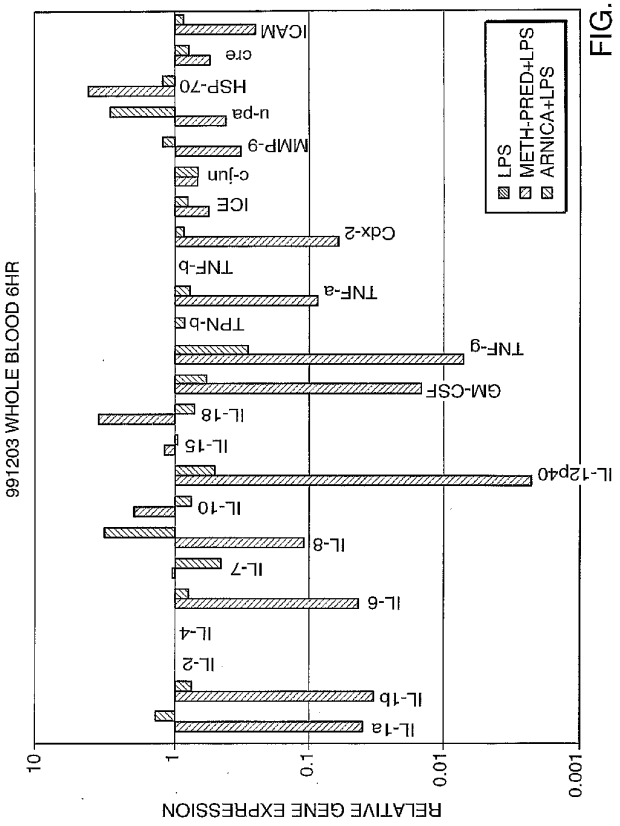

991203 WHOLE BLOOD 6HR
RELATIVE GENE EXPRESSION
10
1
0.1
0.01
0.001
LPS
METH-PRED+LPS
ARNICA+LPS
IL-1a
IL-1b
IL-2
IL-4
IL-6
IL-7
IL-8
IL-10
IL-12p40
IL-15
IL-18
GM-CSF
TNF-g
TPN-b
TNF-a
TNF-b
Cdx-2
ICE
c-jun
MMP-9
u-pa
HSP-70
cre
ICAM

FIG. 22

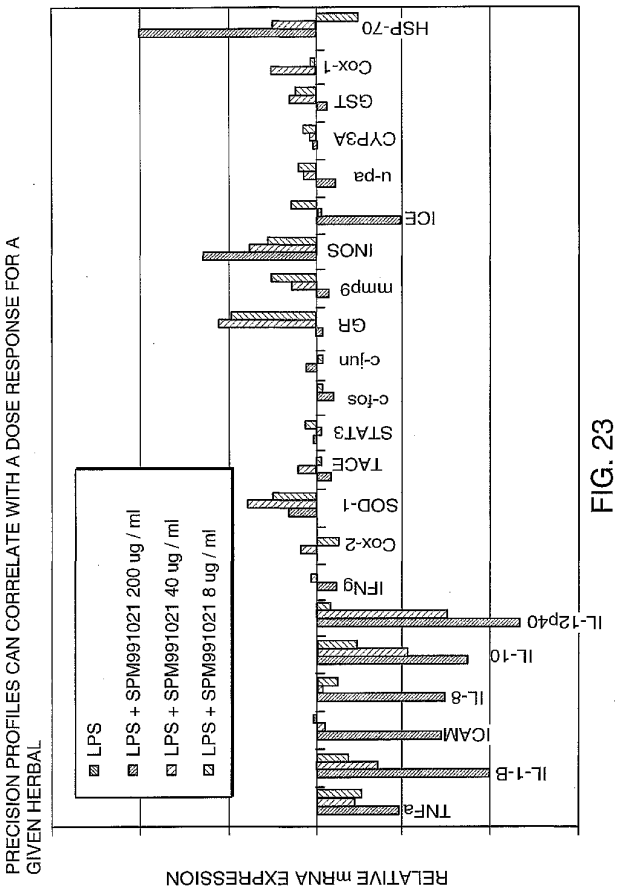

PRECISION PROFILES CAN CORRELATE WITH A DOSE RESPONSE FOR A GIVEN HERBAL
RELATIVE mRNA EXPRESSION
LPS
LPS + SPM991021 200 ug / ml
LPS + SPM991021 40 ug / ml
LPS + SPM991021 8 ug / ml
TNFa
IL-1-B
ICAM
IL-8
IL-10
IL-12p40
IFNg
Cox-2
SOD-1
TACE
STAT3
c-fos
c-jun
GR
mmp9
iNOS
ICE
u-pa
CYP3A
GST
Cox-1
HSP-70

FIG. 23

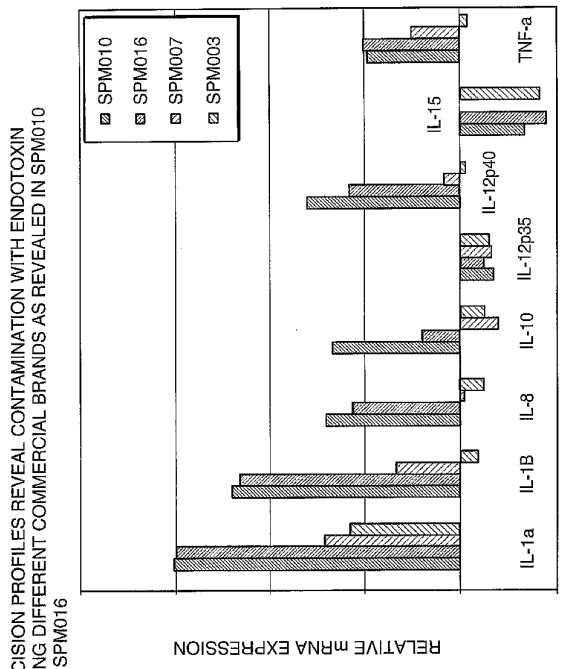

PRECISION PROFILES REVEAL CONTAMINATION WITH ENDOTOXIN
AMONG DIFFERENT COMMERCIAL BRANDS AS REVEALED IN SPM010
AND SPM016
SPM010
SPM016
SPM007
SPM003
RELATIVE mRNA EXPRESSION
IL-1a
IL-1B
IL-8
IL-10
IL-12p35
IL-12p40
IL-15
TNF-a

FIG. 24

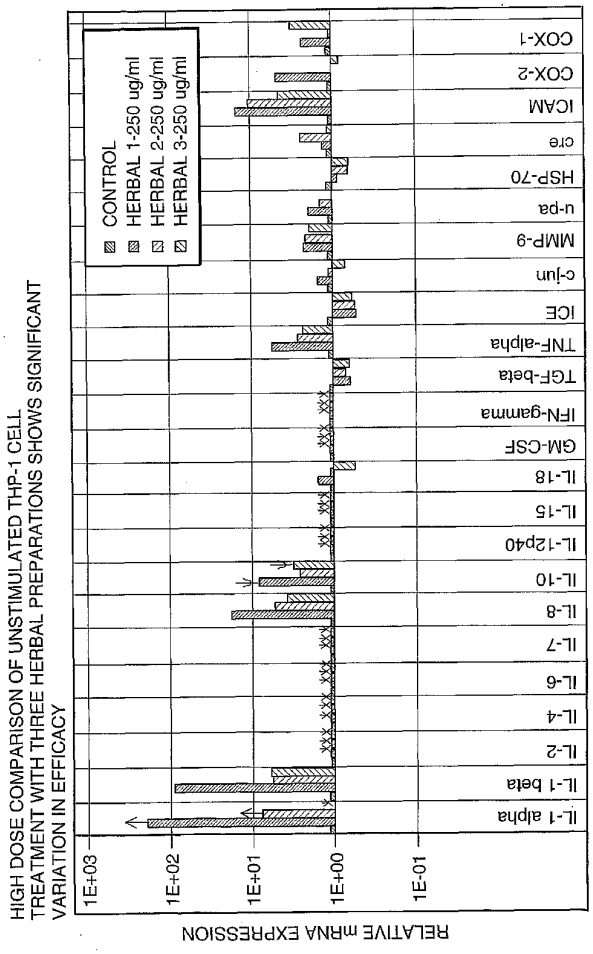

HIGH DOSE COMPARISON OF UNSTIMULATED THP-1 CELL
TREATMENT WITH THREE HERBAL PREPARATIONS SHOWS SIGNIFICANT
VARIATION IN EFFICACY
CONTROL
HERBAL 1-250 ug/ml
HERBAL 2-250 ug/ml
HERBAL 3-250 ug/ml
RELATIVE mRNA EXPRESSION
1E+03
1E+02
1E+01
1E+00
1E-01
IL-1 alpha
IL-1 beta
IL-2
IL-4
IL-6
IL-7
IL-8
IL-10
IL-12p40
IL-15
IL-18
GM-CSF
IFN-gamma
TGF-beta
TNF-alpha
ICE
c-jun
MMP-9
u-pa
HSP-70
cre
ICAM
COX-2
COX-1

FIG. 25a

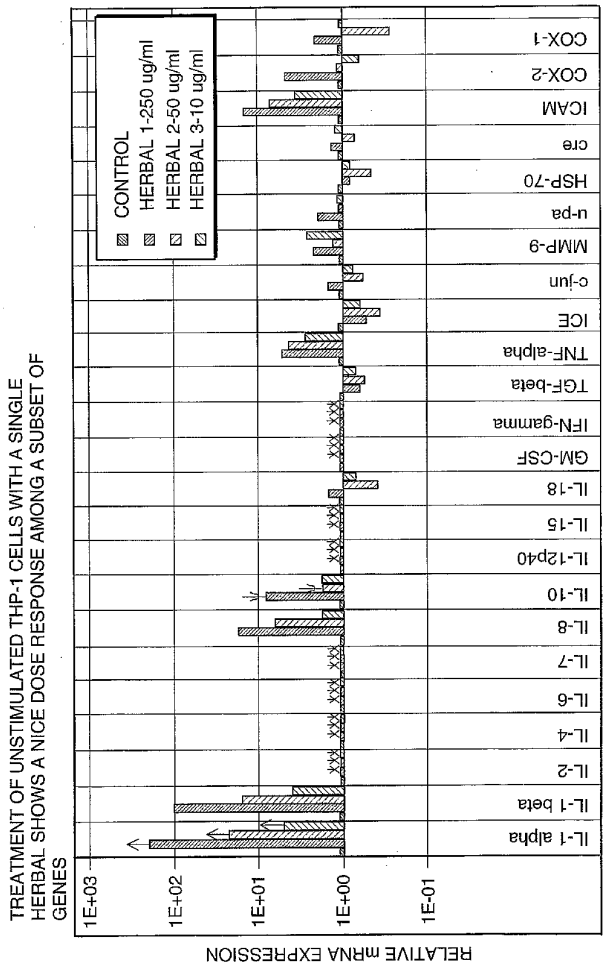

TREATMENT OF UNSTIMULATED THP-1 CELLS WITH A SINGLE HERBAL SHOWS A NICE DOSE RESPONSE AMONG A SUBSET OF GENES
CONTROL
HERBAL 1-250 ug/ml
HERBAL 2-50 ug/ml
HERBAL 3-10 ug/ml
IL-1 alpha
IL-1 beta
IL-2
IL-4
IL-6
IL-7
IL-8
IL-10
IL-12p40
IL-15
IL-18
GM-CSF
IFN-gamma
TGF-beta
TNF-alpha
ICE
c-jun
MMP-9
u-pa
HSP-70
cre
ICAM
COX-2
COX-1
1E+03
1E+02
1E+01
1E+00
1E-01
RELATIVE mRNA EXPRESSION

FIG. 25b

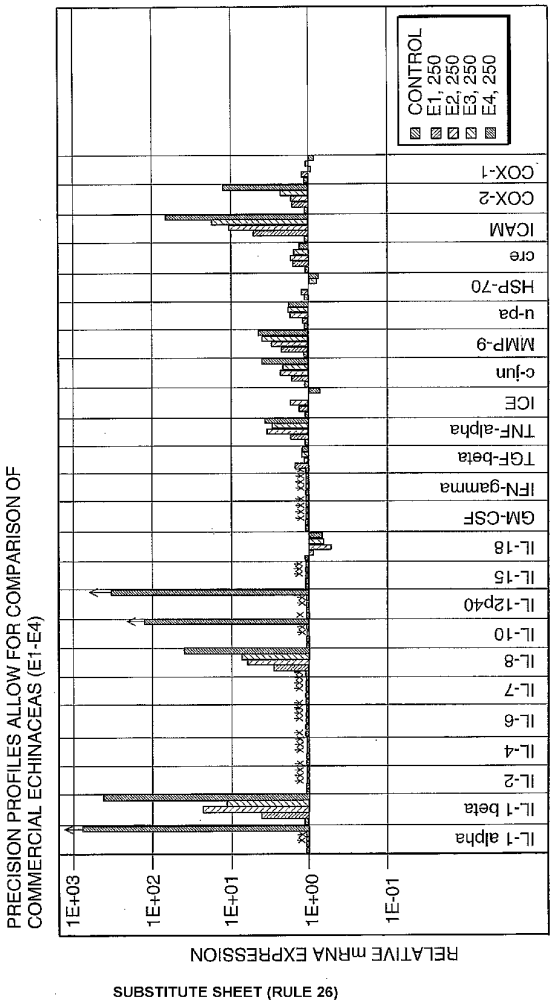

PRECISION PROFILES ALLOW FOR COMPARISON OF COMMERCIAL ECHINACEAS (E1-E4)
RELATIVE mRNA EXPRESSION
1E+03
1E+02
1E+01
1E+00
1E-01
CONTROL
E1, 250
E2, 250
E3, 250
E4, 250
IL-1 alpha
IL-1 beta
IL-2
IL-4
IL-6
IL-7
IL-8
IL-10
IL-12p40
IL-15
IL-18
GM-CSF
IFN-gamma
TGF-beta
TNF-alpha
ICE
c-jun
MMP-9
u-pa
HSP-70
cre
ICAM
COX-2
COX-1

FIG. 25c

【国際調査報告】

INTERNATIONAL SEARCH REPORT

International Application No: PCT/US 00/17846

A. CLASSIFICATION OF SUBJECT MATTER
C12Q1/00,C12Q1/68

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)
C12Q,G01N

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)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 99/04251 A1 (BIO-RAD LABORATORIES INC.) 28 January 1999, abstract, claims. --	1-141
Y	WO 98/24935 A1 (UROCOR, INC.) 11 June 1998, abstract, claims 1,10,24. --	1-141
Y	WO 99/11822 A1 (GENE LOGIC, INC.) 11 March 1999, pages 1,2, claim, fig. 1. --	1-141
Y	WO 94/23023 A1 (UNIVERSITY OF ROCHESTER) 13 October 1994, abstract, pages 1-7.	1-141

☒ Further documents are listed in the continuation of box C.

☒ 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
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"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
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"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
21 November 2000

Date of mailing of the international search report
1, 03, 01

Name and mailing address of the ISA
European Patent Office, P.B. 5818 Patentlaan 2
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Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

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Form PCT/ISA/210 (second sheet) (July 1992)

ANHANG
Zum internationalen Recherchenbericht über die internationale Patentanmeldung Nr.

ANNEX
To the International Search Report to the international Patent Application No.

ANNEXE
Au rapport de recherche international relatif à la demande de brevet international n°

PCT/US 00/17846 SAE 293194

In diesem Anhang sind die Mitglieder der Patentfamilien der im obengenannten internationalen Recherchenbericht angeführten Patentdokumente angegeben. Diese Angaben dienen nur zur Unterrichtung und erfolgen ohne Gewähr.

This annex lists the patent family members relating to the patent documents cited in the above-mentioned search report. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

La présente annexe indique les membres de la famille de brevets relatifs aux documents de brevets cités dans le rapport de recherche international visée ci-dessus. Les renseignements fournis sont donnés à titre indicatif et n'engagent pas la responsabilité de l' Office.

Im Recherchenbericht angeführte Patentdokumente Patent document cited in search report Document de brevet cité dans le rapport de recherche	Datum der Veröffentlichung Publication date Date de publication	Mitglied(er) der Patentfamilie Patent family member(s) Membre(s) de la famille de brevets	Datum der Veröffentlichung Publication date Date de publication
WO A1 9904251	28-01-1999	AU A1 85719/98	10-02-1999
		EP A1 1047931	02-11-2000
		SG A1 48536	17-04-1998
		US A 5922184	13-07-1999
		JP A2 9237419	09-09-1997
		US A 5863473	26-01-1999
WO A1 9824935	11-06-1998	AU A1 55151/98	29-06-1998
		AU B2 722819	10-08-2000
		EP A1 960214	01-12-1999
WO A1 9911822	11-03-1999	AU A1 92171/98	22-03-1999
WO A1 9423023	13-10-1994	AU A1 64931/94	24-10-1994
		JP A2 7132100	23-05-1995
		US A 5639606	17-06-1997
		US A 5876978	02-03-1999
		US A 5643765	01-07-1997
		AU A1 80728/98	04-01-1999
		CN T 1265155	30-08-2000
		EP A2 998581	10-05-2000
		WO A2 9858083	23-12-1998
		WO A3 9858083	11-03-1999

フロントページの続き

(81)指定国 AP(GH,GM,KE,LS,MW,MZ,SD,SL,SZ,TZ,UG,ZW),EA(AM,AZ,BY,KG,KZ,MD,RU,TJ,TM),EP(AT,BE,CH,CY,DE,DK,ES,FI,FR,GB,GR,IE,IT,LU,MC,NL,PT,SE),OA(BF,BJ,CF,CG,CI,CM,GA,GN,GW,ML,MR,NE,SN,TD,TG),AE,AG,AL,AM,AT,AU,AZ,BA,BB,BG,BR,BY,BZ,CA,CH,CN,CR,CU,CZ,DE,DK,DM,DZ,EE,ES,FI,GB,GD,GE,GH,GM,HR,HU,ID,IL,IN,IS,JP,KE,KG,KP,KR,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,MN,MW,MX,MZ,NO,NZ,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,UZ,VN,YU,ZA,ZW

(74)代理人 100119253
弁理士 金山 賢教
(72)発明者 トライオン、ビクター
アメリカ合衆国、コロラド州 ８０５３７、ラブランド、エスダブリュ・フォーティーンス・ストリート ６７７０
(72)発明者 ベビラクゥア、マイケル・ピー
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