Title: TRUNCATED EPIDERMAL GROWTH FACTOR RECEPTOR (EGFRt) FOR TRANSDUCED T CELL SELECTION

Abstract: A non-immunogenic selection epitope may be generated by removing certain amino acid sequences of the protein. For example, a gene encoding a truncated human epidermal growth factor receptor polypeptide (EGFRt) that lacks the membrane distal EGF-binding domain and the cytoplasmic signaling tail, but retains an extracellular epitope recognized by an anti-EGFR antibody is provided. Cells may be genetically modified to express EGFRt and then purified without the immunooactivity that would accompany the use of full-length EGFR immunoactivity. Through flow cytometric analysis, EGFRt was successfully utilized as an in vivo tracking marker for genetically modified human T cell engraftment in mice. Furthermore, EGFRt was demonstrated to have cellular depletion potential through cetuximab mediated antibody dependent cellular cytotoxicity (ADCC) pathways. Thus, EGFRt may be used as a non-immunogenic selection tool, tracking marker, a depletion tool or a suicide gene for genetically modified cells having therapeutic potential.
<table>
<thead>
<tr>
<th>Box No. 1</th>
<th>Nucleotide and/or amino acid sequence(s) (Continuation of item 1c of the first sheet)</th>
</tr>
</thead>
</table>

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:
   a. (means)
      - ☑ on paper
      - ✗ in electronic form
   b. (time)
      - ✗ in the international application as filed
      - ☑ together with the international application in electronic form
      - subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:
   GenCore 6.3: SEQ ID NO:3, 6
**INTERNATIONAL SEARCH REPORT**

**Box No. II** Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III** Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

Group 1: claims 1-11, drawn to a gene encoding a modified endogenous cell-surface molecule, which molecule comprises an extracellular epitope recognized by a known antibody or functional fragment thereof; but which molecule lacks a signaling or trafficking domain, rendering the endogenous cell-surface molecule inert.

Group 2: claims 12-20, drawn to a method of selecting transduced T cells by (a) transducing a population of T cells with a gene encoding a modified endogenous cell-surface molecule, which molecule comprises an extracellular epitope recognized by a known antibody or functional fragment thereof; but which molecule lacks a signaling or trafficking domain, rendering the endogenous cell surface molecule inert.

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

- ☒ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/2 10 (continuation of first sheet (2)) (July 2009)
C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>US 6,790,614 B2 (PYPPIG et al) 14 September 2004 (14.09.2004) col 1, ln 58-67; col 2; ln 1-3, 32-41, 49-51; col 4, ln 40-47; col 9, ln 9-17; col 13, ln 43-57; col 16, ln 56-67; fig 1</td>
<td>1-3</td>
</tr>
<tr>
<td>Y</td>
<td>Li et al. Structural basis for inhibition of the epidermal growth factor receptor by cetuximab. Cancer Cell 2005, 7:301-311; pg 304, left col, para 3; pg 306, left col, para 1</td>
<td>4-5, 7-8, 12-18</td>
</tr>
<tr>
<td>Y</td>
<td>CHAKRAVERTY et al. An inflammatory checkpoint regulates recruitment of graft-versus-host reactive T cells to peripheral tissues. JEM 2006, 203(8):2021-2031; pg 2024, right col, para 1; pg 2029, left col, para 2</td>
<td>4-5, 7-8, 14-16</td>
</tr>
</tbody>
</table>

* Further documents are listed in the continuation of Box C.

** 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 application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"K" document member of the same patent family

Date of the actual completion of the international search: 10 March 2011 (10.03.2011)

Date of mailing of the international search report: 07 APR 2011

Name and mailing address of the ISA/US:

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-272-3280

Authorized officer: Lee W. Young

PCT Facsimile: 571-272-4300
PCT OSP: 571-272-7774

Form PCT/ISA2/10 (second sheet) (July 2009)
The inventions listed as Groups I-II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The inventions of Group I do not include the inventive concept of a method of selecting transduced T cells by (a) transducing a population of T cells with a gene encoding a modified endogenous cell-surface molecule, which molecule comprises an extracellular epitope recognized by a known antibody or functional fragment thereof; but which molecule lacks a signaling or trafficking domain, rendering the endogenous cell-surface molecule inert, as required by Group II.

The inventions of Groups I-II share the technical feature of a gene encoding a modified endogenous cell-surface molecule, which molecule comprises an extracellular epitope recognized by a known antibody or functional fragment thereof; but which molecule lacks a signaling or trafficking domain, rendering the endogenous cell-surface molecule inert. However, this shared technical feature does not represent a contribution over the prior art as as being anticipated by the article titled "Structural basis for inhibition of the epidermal growth factor receptor by cetuximab" by Li et al. (hereinafter 'Li') (Cancer Cell. 2005, 7(4):301-11) that teaches a gene encoding a modified endogenous cell-surface molecule (pg 309, left col, para 4; Domain III), which molecule comprises an extracellular epitope recognized by a known antibody or functional fragment thereof (pg 304, left col, para 3, "The cetuximab Fab fragment binds exclusively to domain III,..."); but which molecule lacks a signaling or trafficking domain, rendering the endogenous cell-surface molecule inert (pg 306, left col, para 1, "To confirm that only domain III is required for the high-affinity interaction of FabC225 with sEGFR, we generated recombinant baculovirus to direct expression and secretion from Sf9 cells of isolated domain III (amino acids 310754 of mature EGFR)"). As said gene was known at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

Groups I-II therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.