DRUG TARGETING
Focusing drug actions at target tissue sites
20.380 S10 workshop
What is drug targeting?


(Wickham, 2003)
Motivation for drug targeting: General

• Many drugs are toxic if delivered systemically:
  • Nonspecific radio/chemotherapeutic drugs
  • Top 6 chemotherapeutics nonspecifically kill proliferating cells
    • …thus lower doses used
    • …in cancer, tumor has time to mutate, leading to development of drug resistant tumors
  • Protein drugs may act specifically on many tissues distal to target tissue
Major approaches for targeted delivery

1) receptor-ligand targeting

2) Pre-targeting

(i) CLEAR UNBOUND "BRIDGING AGENT"

3) Antibody-based targeting

4) "Reverse" targeting
Issues to consider:

• Where is the target molecule expressed?
  • Is it expressed by normal tissues?
  • Is it stably expressed?
  • Can select out evasive tumor cells/viruses


• What is the affinity of binding?
  • Does binding trigger endocytosis? Could be good or bad
  • Immune response to targeting agent

Some healthy tissues can be ablated in an acceptable manner.

Peculiarly acute for mAb targeting:

- t1/2 in humans
- Mouse Ab: <20 min
- Human Ab: 12 d

Lecture 23 Spring 2006
### (1) Receptor-ligand mediated targeting

<table>
<thead>
<tr>
<th>Application</th>
<th>Cellular target</th>
<th>Molecular target</th>
<th>Targeting ligand</th>
<th>Ligand type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-cancer therapy</td>
<td>Various tumor cells</td>
<td>Folate receptor</td>
<td>Folate EGF</td>
<td>Protein ligand for target receptor preferentially expressed on target cells</td>
</tr>
<tr>
<td>Anti-cancer therapy, pulmonary, cardiovascular,</td>
<td>Neovascular tissue</td>
<td>EGF receptor</td>
<td>anti-B-FN antibody</td>
<td>antibody against fibronectin isoform only expressed during embryonic</td>
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<td>and inflammatory diseases</td>
<td></td>
<td></td>
<td></td>
<td>development and in aggressive tumors</td>
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<tr>
<td>Anti-cancer therapy (leukemias and B cell</td>
<td>Endothelial cells</td>
<td>E-selectin P-selectin</td>
<td>sialyl Lewis’</td>
<td>receptor expressed at sites of inflammation</td>
</tr>
<tr>
<td>lymphomas)</td>
<td></td>
<td></td>
<td>receptor</td>
<td></td>
</tr>
<tr>
<td>Anti-cancer therapy (T cell lymphomas)</td>
<td>Transformed B lymphocytes</td>
<td>CD20</td>
<td>Anti-CD20 antibody</td>
<td>Antibody against target cell-surface protein unique to target class of</td>
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<td></td>
<td></td>
<td></td>
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<td>cells (e.g. B cells)</td>
</tr>
<tr>
<td>Anti-cancer therapy (T cell lymphomas)</td>
<td>Transformed T lymphocytes</td>
<td>IL-2Rα (interleukin-2</td>
<td>Anti-IL-2Rα antibody</td>
<td>Antibody against target cell-surface protein not expressed on normal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>receptor a chain</td>
<td></td>
<td>resting cells</td>
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</tbody>
</table>

- **Cytotoxic drugs**
  - AraC
  - Doxorubicin
- **Anti-tumor cytokines**
  - Interleukin-2
  - Interleukin-12

**OVEREXPRESSION ON 95% OVARIAN CARCINOMAS**

**LOSS OF HEALTHY B CELLS OK: BONE MARROW STEM CELL TRANSPLANT (ALLOGENOUS)**
<table>
<thead>
<tr>
<th>Targeting ligands and antibodies</th>
<th>Alternative names (trade name)</th>
<th>Target</th>
<th>Example of tumour target</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-antibody</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>RGD</td>
<td>Cellular adhesion molecules, such as αvβ3-integrin</td>
<td>Vasculature endothelial cells in solid tumours</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>NGR</td>
<td>Aminopeptidase N (CD13)</td>
<td>Vasculature endothelial cells in solid tumours</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Folate</td>
<td>Folate receptor</td>
<td>Cancer cells that overexpress the folate receptor</td>
<td>101, 102</td>
<td></td>
</tr>
<tr>
<td>Transferrin</td>
<td>Transferrin receptor</td>
<td>Cancer cells that overexpress the transferrin receptor</td>
<td>103, 104</td>
<td></td>
</tr>
<tr>
<td>GM-CSF</td>
<td>GM-CSF receptor</td>
<td>Leukaemic blasts</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>Galactosamine</td>
<td>Galactosamine receptors on hepatocytes</td>
<td>Hepatoma</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td><strong>Antibody</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-VEGFR</td>
<td>2C3</td>
<td>Vasculature endothelial growth-factor receptor (FLK1)</td>
<td>Vasculature endothelial cells in solid tumours</td>
<td>105</td>
</tr>
<tr>
<td>Anti-ERBB2</td>
<td>Trastuzumab (Herceptin)</td>
<td>EREB2 receptor</td>
<td>Cells that overexpress the ERBB2 receptor, such as in breast and ovarian cancers</td>
<td>7</td>
</tr>
<tr>
<td>Anti-CD20</td>
<td>Rituximab (Rituxan), ibritumomab tiuxetan (Zevalin)</td>
<td>CD20, a B-cell surface antigen</td>
<td>Non-Hodgkin’s lymphoma and other B-cell lymphoproliferative diseases</td>
<td>8</td>
</tr>
<tr>
<td>Anti-CD22</td>
<td>Epratuzumab, LL2, RFB4</td>
<td>CD22, a B-cell surface antigen</td>
<td>Non-Hodgkin’s lymphoma and other B-cell lymphoproliferative diseases</td>
<td>33, 52</td>
</tr>
<tr>
<td>Anti-CD19</td>
<td>B4, HD37</td>
<td>CD19, a pan-B-cell surface epitope</td>
<td>Non-Hodgkin’s lymphoma and other B-cell lymphoproliferative diseases</td>
<td>49, 52</td>
</tr>
<tr>
<td>Anti-CD33</td>
<td>Gemtuzumab, ozogamicin (Mylotarg)</td>
<td>CD33, a sialo-adhesion molecule, leucocyte differentiation antigen</td>
<td>Acute myeloid leukaemia</td>
<td>37, 67</td>
</tr>
<tr>
<td>Anti-CD33</td>
<td>M195</td>
<td>CD33, a T-cell epitope</td>
<td>Acute myeloid leukaemia</td>
<td>37</td>
</tr>
<tr>
<td>Anti-CD25</td>
<td>Anti-Tac, LMB2</td>
<td>CD25, α-subunit of the interleukin-2 receptor on activated T cells</td>
<td>Hairy-cell leukaemia, Hodgkin’s and other CD25+ lymphoma haematological malignancies</td>
<td>106</td>
</tr>
<tr>
<td>Anti-CD25</td>
<td>Denileukin diftitox (Ontak)</td>
<td>Interleukin-2 receptor</td>
<td>Cutaneous T-cell lymphoma</td>
<td>46, 47</td>
</tr>
<tr>
<td>Anti-HLA-DR10β</td>
<td>Lym1</td>
<td>HLA-DR10β subunit</td>
<td>Non-Hodgkin’s lymphoma and other B-cell lymphoproliferative diseases</td>
<td>32</td>
</tr>
<tr>
<td>Anti-tensin</td>
<td>81C6</td>
<td>Extracellular-matrix protein overexpressed in many tumours</td>
<td>Gial tumours, breast cancer</td>
<td>107</td>
</tr>
<tr>
<td>Anti-CEA</td>
<td>MN-14, F6, A5B7</td>
<td>CEA</td>
<td>Colorectal, small-cell lung and ovarian cancers</td>
<td>28, 108</td>
</tr>
<tr>
<td>Anti-MUC1</td>
<td>HMFG1, BrE3</td>
<td>MUC1, an aberrantly glycosylated epithelial mucin</td>
<td>Breast and bladder cancer</td>
<td>28, 109</td>
</tr>
<tr>
<td>Anti-TAG72</td>
<td>CC49, B72.3</td>
<td>TAG72, oncotel antigen tumour-associated glycoprotein-72</td>
<td>Colorectal, ovarian and breast cancer</td>
<td>28, 110</td>
</tr>
</tbody>
</table>

CEA, carcinoembryonic antigen; GM-CSF, granulocyte-macrophage colony-stimulating factor; LTTs, ligand-targeted therapeutics; NGR, Asn-Gly-Arg tripeptide; RGD, Arg-Gly-Asp tripeptide; TAG72, oncotel antigen tumour-associated glycoprotein-72; VEGFR, vascular endothelial growth-factor receptor.

Targeting agents may bring cargo to specific cell type or just localize the cargo at the tissue area
Example approach: receptor-ligand-mediated targeting to vasculature at sites of inflammation

Mimicking lymphocyte responses to inflammation:

Figure removed due to copyright restrictions.
Example approaches: receptor-ligand-mediated targeting to vasculature

Diagram of mimicking lymphocyte responses to inflammation removed due to copyright restrictions.
(2) Pre-targeting drug delivery with bispecific antibodies

Figure showing schematic of three-step pretargeting radioimmunotherapy from Drugs of the Future journal removed due to copyright restrictions.
See Figure 2 in Lam, L. X. Liu, and Y. Cao. "Pretargeted Radioimmunotherapy, A Potential Cancer Treatment." Drugs of the Future 28, no. 2 (2003).

(Cao and Lam, 2003)
(3) Antibody-based targeting

General structure of IgA, IgE, IgD, IgG:
Generation of monoclonal antibodies against selected molecular targets

Figure showing the standard procedure for development of monoclonal antibodies removed due to copyright restrictions. See Figure 4-12 from "Immunology: Understanding the Immune System" by Klaus D. Elgert (1996).

Monoclonal: All Abs Are Identical

Pros of Ab Targeting:

1. Specificity
2. High Affinity Possible

Typically

$K_D \approx 0.1-100 \text{ nM}$

$t_{1/2} (37^\circ C) \approx 30-60 \text{ min}$

(Elgert 1996)
Synthesizing antibodies which avoid recognition by the immune system

Figure 2 | Antibodies and antibody fragments. Targeting antibodies are normally monoclonal immunoglobulin G (IgG) (Aa) or IgG fragments (B–D). Fab’ (B), (C) or Fab’ (C) fragments can be made by enzymatic cleavage of the whole monoclonal antibody (mAb) (Aa) or by molecular biological techniques — for example, Fab’ (C), scFv (Da), bivalent (Db) or recombinant fragments (Dc). mAbs that are made from the traditional hybridoma technique are murine in origin. Recent developments have led to improved techniques for the production of chimeric, humanized or fully human antibodies or fragments (Ab–d). VH, variable heavy chain; VL, variable light chain.

(Allen 2002) 20.380 drug targeting workshop

Strategies for conjugation of antibodies to biomaterials

Figures showing pepsin and papain enzyme digestion of antibodies removed due to copyright restrictions.
Role of nanoparticle carriers in promoting multivalent interactions with targets

1) **Chemotaxis:** Migration ‘up’ concentration gradients of chemoattractant

2) **Antigen loading and activation**

3) ** Trafficking to lymph nodes**

4) **Activation of naïve T cells in the lymph nodes**

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(4) ‘Reverse targeting’ to mimic infection site recruitment

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1) Attraction to sites of infection

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Lecture 23 Spring 2006
Electron micrograph images of dendritic cells and T-cells attracted to a tissue site removed due to copyright restrictions.
IMPACT OF TARGETING IN VIVO
PASSIVE TARGETING OF TUMORS:
Enhanced permeation and retention (EPR) effect in tumors:

1. Leaky, chaotic vasculature
   Gaps in endothelium
   50-700 nm

2. Blocked lymphatic drainage

3. Elevated interstitial fluid pressure

Lymphatics

Tumor

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Key to remember: vasculature is not the only barrier to diffusion in vivo

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100-NPs

25-NPs

Enhanced permeation and retention (EPR) effect in tumors:

(Lammers et al. Neoplasia 8 788-795 (2006))
Results from mAb-targeting: targeting tumors

Fig. 4. Immunoliposomes binding to the surface of an ovarian carcinoma cell. This electron micrograph depicts a human OVCAR-3 cell taken from the peritoneal cavity of nude mice after injecting the animals intraperitoneally with OV-TL3-Fab'-immunoliposomes. A more detailed analysis of the cell-immunoliposome interaction showed very little endocytic uptake. A search was started to identify endocytosis inducing antibodies. mAB with human ovarian cancer cell specificity were identified (e.g., mAB 425). These 425 immunoliposomes loaded with DTA and a pH-dependent fluorogen (dABF-7) were tested in vivo [15,17].

Results from mAb-targeting: Targeting tumors

ONE MORE IDEA, time permitting: CELL-MEDIATED TARGETING
lymphocyte homing to target tissue sites: the basis of adoptive T-cell therapy for cancer

adoptive T-cell therapy

Imaging the trafficking of tumor-specific T-cells following i.v. injection:


Concept: Combine adoptive cell therapy with nanoparticle delivery

Target tissue sites:
- ?
- tumors
- gut
- skin
- bone marrow
- lymphoid organs
How to stably link nanoparticles to the surfaces of living cells?

**coupling to free surface thiols**

**in situ PEGylation**

**T-Cell PLASMA MEMBRANE**

**nanoparticles**

**T-cell overlay**

**2 µm**
Images showing nanoparticle accumulation in tumors is more effective when carried there by T-cells have been removed due to copyright restrictions.
T-cells “armed” with particles releasing cytokine IL-15 exhibit greatly enhanced antitumor activity

Figure showing tumor and T-cell imaging of mice removed due to copyright restrictions.