

Supplementary file

**Table S1.** Representative examples of Ab-based antitumor proteins for cancer immunotherapy recently reported in the literature.

| TARGET Ag             | TARGET TUMOR                                             | Ab FORMAT                                                                   | FUSED PROTEIN OR CONJUGATED MOIETY   | DRUG NAME               | Ab PROPERTY/ INTRINSIC ACTIVITY                            | ADDED PROPERTY                            | RELEVANT COMMENT                                                 | Ref. | a | b | c | d | e | f    |
|-----------------------|----------------------------------------------------------|-----------------------------------------------------------------------------|--------------------------------------|-------------------------|------------------------------------------------------------|-------------------------------------------|------------------------------------------------------------------|------|---|---|---|---|---|------|
| 5T4                   | Pancreatic, non-small-cell lung (NSCLC) and renal cancer | Murine Fab                                                                  | Mutated Staphylococcal Enterotoxin A | Naptumomab estafenatox  | Tumor targeting                                            | Superantigen-mediated T-cell cytotoxicity | Stable disease in 36% of NSCLC patients                          | [1]  |   |   |   |   |   |      |
| Carbonic anhydrase IX | Non-metastatic renal cell carcinoma                      | Chimeric (Mouse x Human) IgG1 (C <sub>H</sub> 2-C <sub>H</sub> 3 truncated) | Human TNF                            | c(G250)-TNF             | Tumor targeting                                            | Improved survival                         | Synergy observed when administered in combination w/IFN $\gamma$ | [2]  |   |   |   |   |   |      |
| CCR4                  | Adult T-cell Leukemia/ Lymphoma                          | Defucosylated humanized IgG1                                                | none                                 | Mogamulizumab (KW-0761) | Tumor targeting                                            | Increased ADCC                            | NK dependent                                                     | [3]  |   |   |   |   |   |      |
| CD4                   | Cutaneous T-cell lymphoma                                | Human IgG1                                                                  | none                                 | Zanolimumab             | Receptor downmodulation, alteration of signal transduction | ADCC                                      | Phase III trial results due on Feb 2011                          | [4]  |   |   |   |   |   |      |
| CD20                  | Non-Hodgkin's Lymphoma (NHL)                             | Murine IgG2a                                                                | Isotope 131Iodine                    | 131I-Tositumomab        | Tumor targeting                                            | Radiation delivery, bystander effect      | 93% complete remission, combined w/chemotherapy                  | [5]  |   |   |   |   |   | 2003 |

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| TARGET Ag    | TARGET TUMOR                                     | Ab FORMAT                                   | FUSED PROTEIN OR CONJUGATED MOIETY | DRUG NAME                            | Ab PROPERTY/ INTRINSIC ACTIVITY | ADDED PROPERTY                       | RELEVANT COMMENT                                                                         | Ref. | a | b | c | d | e | f    |
|--------------|--------------------------------------------------|---------------------------------------------|------------------------------------|--------------------------------------|---------------------------------|--------------------------------------|------------------------------------------------------------------------------------------|------|---|---|---|---|---|------|
| CD20 (Cont.) | NHL                                              | Murine IgG1                                 | Isotope <sup>90</sup> Yttrium      | <sup>90</sup> Y-Ibritumomab tiuxetan | Tumor targeting                 | Radiation delivery, bystander effect | 72% complete remissions after Rituximab with chemotherapy                                | [6]  |   |   |   |   |   | 2002 |
|              | Lymphoma model                                   |                                             | 41-BBL (CD137L)                    | 4-1BBL/anti-CD20                     | Tumor targeting                 | Increased T-cell cytotoxicity        | Combined w/anti-CD3-CD20 diabody, leads to tumor inhibition                              | [7]  |   |   |   |   |   |      |
|              | Lymphoma model                                   | Recombinant deimmunized murine V Human IgG1 | IL-2                               | DI-Leu16(IL-2)                       | Tumor targeting                 | Enhanced ADCC                        | Activity partially dependent on FcR binding                                              | [8]  |   |   |   |   |   |      |
|              | Lymphoma model                                   | Chimeric (Mouse x Human) IgG3               | IFN- $\alpha$                      | Anti-CD20-IFN- $\alpha$              | Tumor targeting                 | Increased apoptosis, but not ADCC    | Eradication of tumor xenografts required IFN receptor expression                         | [9]  |   |   |   |   |   |      |
| CD21         | Prostate model                                   | Human IgG1 Fc                               | CR2                                | CR2-Fc                               | Complement activator            | Enhanced CDC and ADCC                | In combination w/anti-MUC1 Ab                                                            | [10] |   |   |   |   |   |      |
| CD22         | Hairy cell leukemia                              | dsFv                                        | Pseudomonas Exotoxin A             | CAT-8015, HA22                       | Tumor targeting                 | Transcription inhibition             | Complete remission in 43%, overall response in 79%                                       | [11] |   |   |   |   |   |      |
|              | Follicular NHL and diffuse large B-cell lymphoma | dsFv IgG4                                   | Calcicheamin                       | Inotuzumab ozogamicin                | Tumor targeting                 | DNA cleavage                         | Objective response rate 68% for follicular NHL and 15% for diffuse large B-cell lymphoma | [12] |   |   |   |   |   |      |

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| TARGET Ag  | TARGET TUMOR                            | Ab FORMAT                     | FUSED PROTEIN OR CONJUGATED MOIETY                        | DRUG NAME             | Ab PROPERTY/ INTRINSIC ACTIVITY   | ADDED PROPERTY                                            | RELEVANT COMMENT                                                                            | Ref. | a | b | c | d | e | f    |
|------------|-----------------------------------------|-------------------------------|-----------------------------------------------------------|-----------------------|-----------------------------------|-----------------------------------------------------------|---------------------------------------------------------------------------------------------|------|---|---|---|---|---|------|
| CD30       | Lymphoma model                          | scFv plus Fc of human IgG1    | IL-2                                                      | HRS3scFv-Fc(IL-2)     | Tumor targeting                   | Enhanced immunity                                         | IFN- $\gamma$ secretion in SCID mice                                                        | [13] |   |   |   |   |   |      |
|            | Lymphoma model                          | scFv                          | pRibonuclease                                             | Ber-H2-scFv-hpRNase   | Tumor targeting                   | Protein synthesis inhibition                              | Anti-tumor activity                                                                         | [14] |   |   |   |   |   |      |
|            | Systemic anaplastic large-cell lymphoma | Chimeric (Mouse x Human) IgG1 | Monomethyl auristatin E                                   | Brentuximab vedotin   | Tumor targeting                   | Disrupt microtubule polymerization                        | 87% objective response rate and 57% complete remissions<br>BLA submitted to FDA in May 2011 | [15] |   |   |   |   |   |      |
| CD33       | Acute Myeloid Leukemia (AML)            | Humanized IgG4                | Calicheamicin                                             | Gemtuzumab ozogamicin | Tumor targeting                   | DNA cleavage                                              | Withdraw from the US market in 2010                                                         | [16] |   |   |   |   |   | 2000 |
| CD64       | AML                                     | Humanized scFv                | hRNase Angiogenin                                         | Unknown               | Tumor targeting                   | tRNA-specific RNase-mediated protein synthesis inhibition | Prolonged serum half-life                                                                   | [17] |   |   |   |   |   |      |
| Ang1 and 2 | Tumor Angiogenesis                      | Fc fusion                     | Peptide inhibitor of angiopoietin-1 and -2 receptor, Tie2 | AMG 386               | Extended <i>in vivo</i> half-life | Tumor angiogenesis inhibition                             | Evidence of antitumor activity                                                              | [18] |   |   |   |   |   |      |
| ED-A       | Renal cell cancer mouse model           | Fab diabody                   | IL-2                                                      | F8-(IL-2)             | Directed cytokine targeting       | Increased leukocyte infiltration                          | 2 out of 7 complete cures, in combination with sunitinib                                    | [19] |   |   |   |   |   |      |
|            | F9 murine teratocarcinoma               | ScFv                          | IL-12                                                     | F8-IL-12              | Directed cytokine targeting       |                                                           |                                                                                             | [20] |   |   |   |   |   |      |

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|-----------|------------------------------------|-----------------------|------------------------------------|-----------------------|---------------------------------|---------------------------------------------------------|---------------------------------------------|------|---|---|---|---|---|---|
| ED-B      | F9 and A431 mouse models of cancer | Human IgG SIP         | Porphyrin-based photosensitiser    | Unknown               | Tumor targeting                 | Light irradiation-mediated tumor vasculature disruption | NK-mediated long-lasting complete responses | [21] |   |   |   |   |   |   |
|           | F9 and C51 mouse models of cancer  | Human ScFv            | GM-CSF                             | L19-(GM-CSF)          | Tumor targeting                 | CD8 <sup>+</sup> T-cell involvement                     | Anti-tumoral and anti-metastatic effect     | [22] |   |   |   |   |   |   |
|           | F9 and C51 mouse models of cancer  | Human ScFv            | IL-15                              | L19-(IL15)            | Tumor targeting                 | CD8 <sup>+</sup> T-cell involvement                     | Anti-tumoral and anti-metastatic effect     | [22] |   |   |   |   |   |   |
|           | F9 mouse model of cancer           | Human ScFv            | IL-12                              | L19-(IL-12)           | Tumor targeting                 | Targeting IL-12 to tumor site                           | Synergy w/ L19-TNFa                         | [23] |   |   |   |   |   |   |
|           | Human tumor xenograft              | Humanized IgG1        | IL-12                              | huBC1-IL-12           | Incresased binding affinity     | Targeting IL-12 to tumor site                           | Tumor size and metastases reduction         | [24] |   |   |   |   |   |   |
| EGFR      | Human tumor xenograft              | C225 Ab-derived scFv  | HLA-A2-peptide complexes           | scFv- MHC-peptide     | Tumor targeting                 | Ag presentation bypass                                  | CTL-dependent lysis                         | [25] |   |   |   |   |   |   |
| EpCAM     | Epithelial carcinomas              | Humanized IgG1        | IL-2                               | EMD 273066 (huKS-IL2) | Tumor targeting                 | ADCC and increased immunological activity               | Chills, rigor, anemia as adverse events     | [26] |   |   |   |   |   |   |
|           | Murine Lewis lung cancer           | Humanized IgG1        | IL-2/IL-12                         | KS-(IL-12/IL-2)       | Tumor targeting                 | Induction of IFN-g secretion                            | Similar to immunocytokine combination       | [27] |   |   |   |   |   |   |
|           | Xenograft colon cancer model       | Murine heterominibody | mGM-CSF/IL-2                       | DCH-GM-CSF/IL2        | Tumor targeting                 | T-cell and PBMC mediated cytotoxicity                   | No GM-CSF/IL2 synergy observed              | [28] |   |   |   |   |   |   |

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|-----------------|-----------------------------------|----------------------|------------------------------------|-----------------------------|----------------------------------------|----------------------------------------------------------------------|---------------------------------------------------------------------------------------|------|---|---|---|---|---|---|
| EpCAM (Cont.)   | Gastric cancer                    | Human heterominibody | hGM-CSF/IL-2                       |                             | Tumor targeting                        | PBMC mediated cytotoxicity <i>in vitro</i> & T-cell redirected lysis |                                                                                       | [29] |   |   |   |   |   |   |
|                 | Metastatic melanoma model         | C215 Ab-derived Fab  | SEA                                | C215Fab-SEA                 | Tumor targeting                        | Superantigen-directed T-cell activation                              | Synergy observed when administered in combination w/docetaxel or INF- $\alpha$        | [30] |   |   |   |   |   |   |
| FAP             | FAP-expressing HT1080 cell line   | scFv36               | 41-BBL (CD137L)                    | scFv36-4-1BBL               | Tumor targeting                        | T-cell co-stimulation                                                | In combination w/bispecific antibodies                                                | [31] |   |   |   |   |   |   |
|                 |                                   | ScFv                 | mGITR Ligand                       | Anti-FAP-mGITRL             | Cancer-associated fibroblast targeting | T-cell activation                                                    | Overcome T-reg supression                                                             | [32] |   |   |   |   |   |   |
|                 | Tumor xenograft mouse model       | scFv                 | TNF- $\alpha$                      | Anti-FAP-TNF dimer          | Tumor targeting                        | TNF-mediated macrophage recruitment into tumor tissue                | Combination w/anti-FAP-IL-8 increased antitumoral activity in mouse xenografts models | [33] |   |   |   |   |   |   |
|                 | Tumor xenograft mouse model       | scFv                 | IL-8                               | Anti-FAP-IL8                | Tumor targeting                        | PMN chemoattraction                                                  | Combination w/anti-FAP-TNF increased antitumoral activity in mouse xenografts models  | [33] |   |   |   |   |   |   |
| G <sub>D2</sub> | Relapsed/refractory neuroblastoma | Humanized IgG1       | IL-2                               | Hu 14.18-(IL-2)/ EMD 273063 | Tumor targeting                        | Increased ADCC and immunological activity                            | Stable disease, 21.7% CR rate in patients with minimal disease                        | [34] |   |   |   |   |   |   |
|                 |                                   | Humanized IgG1       | GM-CSF                             | Hu 14.18-(GM-CSF)           | Tumor targeting                        | Increased PMN-mediated ADCC                                          | Mac-1 dependent ADCC                                                                  | [35] |   |   |   |   |   |   |

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|-----------|----------------------------------|----------------|------------------------------------|-------------------------------------|-----------------------------------------|---------------------------------------|----------------------------------------|------|---|---|---|---|---|---|
| HER2/neu  | Breast cancer                    | Humanized IgG1 | DM1                                | Trastuzumab emtansine               | Microtubule disrupting agent            | Activity on refractory tumor cells    | In Phase II, ORR 39.5%                 | [36] |   |   |   |   |   |   |
|           | Tumor xenografts mouse model     | ScFv-Fc        | IL-2                               | HFI                                 | Tumor targeting                         | Improved ADCC <i>in vitro</i>         | Reduction of tumor growth              | [37] |   |   |   |   |   |   |
|           |                                  | Human IgG3     | IL-2                               | anti-HER2/ <i>neu</i> IgG3-(IL-2)   | Tumor targeting                         | Immune stimulation                    | Tumor growth retardation, protection   | [38] |   |   |   |   |   |   |
|           |                                  | Human IgG3     | GM-CSF                             | anti-HER2/ <i>neu</i> IgG3-(GM-CSF) | Tumor targeting                         | Enhanced Th1 and Th2 immune response  | Tumor growth retardation, protection   | [38] |   |   |   |   |   |   |
|           |                                  | Human IgG3     | IL-12                              | anti-HER2/ <i>neu</i> (IL-12)       | Tumor targeting                         | Involvement of T- and NK cells        | Tumor growth retardation, protection   | [39] |   |   |   |   |   |   |
|           |                                  | ScFv           | TNF                                | scFv23/TNF                          | Tumor targeting                         | Increased apoptosis induction         | Induces TNFR expression on tumor cells | [40] |   |   |   |   |   |   |
|           |                                  | ScFv           | hRNase                             | Erbicin-hRNase                      | Her2 epitope different from trastuzumab | Signalling pathway inhibition         | no cardiotoxicity                      | [41] |   |   |   |   |   |   |
|           |                                  | ScFv-Fc        | none                               | Erbicin-hcAb                        | Her2 epitope different from trastuzumab |                                       | no cardiotoxicity                      | [41] |   |   |   |   |   |   |
|           | Human breast tumor cell lines    | Human IgG3     | C5a                                | Anti-HER2/ <i>neu</i> IgG3(C5a)     | Specific cytotoxicity                   | Increased PMN survival and activation | Multiple mechanisms                    | [42] |   |   |   |   |   |   |
|           | HER2 <sup>+</sup> melanoma model | scFv           | aGalCer-loaded CD1d                | sCD1d-anti-HER2                     | Tumor targeting                         | iNKT activation                       | Reduced metastases and tumor size      | [43] |   |   |   |   |   |   |

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|-------------------|---------------------------------------------------|------------------------------|------------------------------------|---------------------------|------------------------------------------|-----------------------------------------|----------------------------------------------|------|---|---|---|---|---|---|
| HLA class II/CD89 | Lymphoma                                          | Bispecific scFv              | none                               | FcaRI x HLA class II      | Tumor/effector targeting                 | PMN recruitment                         | Cytotoxic to resistant primary tumor cells   | [44] |   |   |   |   |   |   |
| IL-13Ra2          | Glioma tumor model                                | scFv                         | PE38                               | anti-IL-13Ra2 (scFv)-PE38 | IL-13Ra2 overexpressing-tumors targeting | Transcription inhibition                | Specific anti-tumor activity in mouse models | [45] |   |   |   |   |   |   |
| Lewis Y           | Rat brain tumor model                             | Chimeric (Mouse x Human) IgG | Doxorubicin                        | BR96-DOX                  | Tumor targeting                          | Standard chemotherapeutic drug delivery | No better than free drug                     | [46] |   |   |   |   |   |   |
| MEL (gp240)       | Melanoma                                          | scFv                         | TNF                                | scFvMEL/TNF               | Tumor targeting                          | Inhibition of SAPK/JNK pathway          | Complete regression in athymic mice          | [47] |   |   |   |   |   |   |
| MUC-1             | Mammary cancer                                    | scFv-Fc of human IgG1        | IL-2                               | C595 scFv-Fc(IL-2)        | Tumor targeting                          | NK activation                           |                                              | [48] |   |   |   |   |   |   |
| NCR ligands       | Prostate cancer                                   | Human Fc IgG1                | NKp30                              | NKp30-Ig                  | Binding to NK targets                    | Macrophage-mediated lysis               | Suppression of tumor development             | [49] |   |   |   |   |   |   |
| OC183B2           | Ovarian cancer                                    | Murine scFv                  | IL-2                               | IL-2-183B2-scFv           | Tumor targeting                          | IL-2 delivery to tumor site             | Biologically active                          | [50] |   |   |   |   |   |   |
| PMSA              | Prostate cancer                                   | MoAb                         | antimicrobial KLA                  | MLN591-KLA                | Tumor targeting                          | apoptosis induction                     | Increased cytotoxicity                       | [51] |   |   |   |   |   |   |
| Tenascin-C        | Tumoral angiogenesis Glioblastoma xenograft model | Human Ig                     | IL-2                               | F16-(IL-2)                | Tumor targeting                          | Leukocyte recruitment into tumors       | 100% CR in mice                              | [52] |   |   |   |   |   |   |
|                   | Gliomas and lung tumors                           | scFv                         | IL-2                               | scFv(G11)-(IL-2)          | Tumor targeting                          | IL-2 delivery                           | Strong staining of human lung tumor sections | [53] |   |   |   |   |   |   |

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|----------------------|------------------------------------|-------------------------------|------------------------------------|-------------------|---------------------------------|-----------------------------------|--------------------------------------------------------------------------------------------------------|------|---|---|---|---|---|---|
| Tissue factor        | Uterine serous papillary carcinoma | Fc fusion                     | factor VII                         | hI-conI           | Higher antigen affinity         | ADCC                              | Strong staining of human ovarian tumor sections                                                        | [54] |   |   |   |   |   |   |
| Transferrin Receptor | Several                            | Chimeric (Mouse x Human) IgG3 | Avidin                             | Anti-hTfR IgG3-Av | Tumor targeting                 | Increased receptor degradation    | Improved with Gambogic acid                                                                            | [55] |   |   |   |   |   |   |
| "TNT"                | Solid tumors                       | Murine IgG                    | Murine 41-BBL (CD137L)             | TNT-3/CD137L      | Targeting of necrotic regions   | T-cell co-stimulation             | T-cell dependent tumor regression                                                                      | [56] |   |   |   |   |   |   |
| VEGF                 | Angiogenesis                       | Fc fusion                     | ECD of VEGFR-1 and -2              | Aflibercept       | Targeting tumor vasculature     | Extended <i>in vivo</i> half-life | Modest activity in non-small lung cell carcinoma. Promising results in metastatic colon adenocarcinoma | [57] |   |   |   |   |   |   |

Ag, antigen. Ab, antibody. Ref, reference. a to f refer to development status: a, in vitro. b, in vivo. c, d and e, clinical studies on Phase I, II and III, respectively. f, FDA approved.

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