TNF receptor superfamily-based targeted cancer immunotherapy

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Chapter 8

Future Perspectives
Currently, agonistic antibodies remain the preferred drug format for therapeutic activation of TNFRSF receptors in oncology. This relates to the fact that there is broad and longstanding experience in the development, production and approval of therapeutic antibodies in oncology. Moreover, recombinant soluble TNFSF ligands were shown to be rather unstable, have low serum half-life (~10–30 min) and appear to be rapidly cleared by the kidneys, thus probably requiring repeated or prolonged infusion regimes. In comparison, antibodies have far superior pharmacokinetics. Of note; size-exclusion chromatography indicates that our scFv:TNFSF-based fusion proteins are likely to be threefold bigger in molecular size that their corresponding untargeted soluble-TNFSF ligand counterparts. However, it remains to be studied whether this increased molecular size indeed translates into improved serum half-life.

Accordingly, various agonistic DR4 antibodies (e.g. mapatumumab) and DR5 antibodies (tigatuzumab, conatumumab and lexatuzumab) aiming to induce apoptosis in cancer cells have been or currently are in clinical trials. Agonistic anti-TRAIL-receptor antibodies mimic the natural receptor ligand TRAIL in binding to DR4 or DR5 which are expressed on the membranes of many types of malignant cells, thereby activating signal transduction pathways that may result in tumor cell apoptosis and a reduction in tumor growth. Mapatumumab is a fully human, agonist antibody that activates the cell death pathway in tumor cells by specifically binding to DR4 with high affinity. Tigatuzumab (aka CS-1008) is a humanized IgG1 antibody that acts as a DR5 agonist and also exerts TRAIL-like activity. However, despite promising preclinical results, few patients responded to treatment with DR4 or DR5-agonistic antibodies. This may be due to intrinsic TRAIL resistance within primary human cancers or to insufficient agonistic activity of the respective DR4/DR5-targeting drug. For instance, current dimeric DR5 antibodies appear to have only a moderate agonistic activity and require secondary cross-linking e.g. via Fcγ receptor-binding to gain full agonistic activity. Indeed, it has been demonstrated that Fcγ receptors (FcγR)-mediated crosslinking increases the cancer-cell-killing activity of TRAIL-R2-specific antibodies in vivo.

Of note, recently the laboratory of Prof. Henning Walczak reported that even in the presence of high numbers of FcγR-expressing immune cells, as found in ovarian cancer ascites, conatumumab-induced apoptosis was not significantly enhanced. Serendipitously, this group discovered a striking synergy between conatumumab and dulanermin (a clinical-grade non-tagged recombinant form
of sTRAIL) in killing cancer cells. The observed increased killing efficiency was due to enhanced DISC formation upon concomitant binding of dulanermin and conatumumab to DR5. The synergy of conatumumab with sTRAIL was further enhanced by combination with the proteasome inhibitor bortezomib or a second mitochondrial-derived activator of caspases (SMAC) mimetic. Importantly, primary human hepatocytes were resistant to this combination of drugs. We hypothesize that this remarkable enhancement of pro-apoptotic activity may be further increased by combining our bsAb DR5xMCSP with dulanermin. Alternatively, even more potent enhancement of DR5-agonistic therapy may be achieved when combining bsAb DR5xMCSP with an appropriate tumor-directed scFv:sTRAIL fusion protein instead of dulanermin.

Obviously, resistance to TRAIL-induced apoptosis in cancer cells remains a clinical challenge. Better knowledge of the molecular and cellular mechanisms of TRAIL resistance is critical for the successful application in cancer therapy of TRAILR agonists in any shape or forms. In general, combinatorial approaches appear necessary to enhance the efficacy death receptor-directed cancer therapy. In this respect, several agents have been described that can upregulate TRAIL-receptors on cancer cells, sensitize for TRAIL or overcome intrinsic resistance mechanisms⁴,⁵. Therapies combining TRAIL formulations, DR4 or DR5 agonistic antibodies with other anticancer therapy such as cytotoxic agents and targeted agents like small inhibitory molecules and anticancer antibodies are currently being evaluated in clinical trials.

Several factors have been considered as crucial for determining the efficacy of antibodies, including selection of optimal antigens targets as well as the affinity, avidity and molecular structure of antibodies. Numerous approaches have explored to enhance the anticancer activity of antibodies, such as direct conjugation with various cytotoxic compounds (e.g. bacterial toxin and cytotoxic drugs), combinations of anticancer antibodies and the use of bispecific antibodies (bsAbs). Because of their dual specificities, bsAbs can be used to redirect populations of preselected immune effector cells to attack cancer cells, irrespective of their intrinsic specificity. Such bsAbs have been developed already decades ago, but have entered the clinic only recently. Blinatumomab was the first bispecific antibody approved by FDA in 2014 for the treatment of patients with Philadelphia chromosome-negative precursor B-cell acute lymphoblastic leukemia (B-cell ALL), an uncommon form of ALL. Blinatumomab (Blincyto) is a so-called
bi-specific T-cell engagers (BiTEs) that specifically targets CD19 present on B cells. Its molecular format is relatively simple, which is just two different scFv’s fused in tandem to form a single chain bispecific antibody. However, its promising clinical may promote the clinical development of a host of more advanced bi-specific antibodies formats that have been developed world wide. For example, in Chapter 3, we reported on tetravalent bsAb MCSPxDR5, comprising the epitope-binding domains of the agonistic anti-DR5 antibody tigatuzumab and the high-affinity anti-MCSP antibody 9.2.27 in tandem and supplemented with a full-length human IgG1 effector domain. The IgG1-Fc region of MCSPxDR5 was able to recruit Fcy-receptor-expressing immune cells (e.g. natural killer cells, macrophages, dendritic cells) and induce antibody-dependent cellular cytotoxicity (ADCC) towards targeted tumor cells. Thus, MCSPxDR5 has dual and potentially synergistic tumoricidal action: direct activation of TRAIL death receptors targeted to the melanoma cells by virtue of its tumor-specific anti-MCSP domain and ADCC by the recruited immune cells through its IgG1 Fc domain. Using this approach we were the first to demonstrate that bsAb MCSPxDR5 can direct the pro-apoptotic function of the anti-DR5 antibody to melanoma cells. This bispecific antibody approach may provide a new avenue to unlock the therapeutic potential of DR5-targeted cancer therapy and may be of value for the targeted treatment of melanoma and other MCSP-expressing malignancies we reported on bsAb MCSPxDR5.

Very recently, results of a comparable bsAb, designated RG7386, were published that largely corroborate our results on bsAb MCSPxDR5. RG7386 is a tetravalent bispecific antibody directed at both FAP and TRAIL-R2. RG7386 triggered tumor cell apoptosis in vitro and in vivo in preclinical tumor models with FAP-positive stroma. RG7386 had single-agent activity against FAP-expressing malignant cells, due to cross-binding of FAP and DR5 across tumor cells. RG7386 antitumor efficacy was independent of FcR cross-linking, and superior to conventional DR5 antibodies. The antitumor activity of was further enhanced when combined with irinotecan or doxorubicin. Compared to RG7386, our bsAb MCSPxDR5 appears to have the selective benefit that tumor-localized FcR-expressing immune effector cells can add ADCC and additional a crosslinking effect to its antitumor effect. Taken together, these data indicate that tumor-directed TRAIL-R agonistic bsAbs may be a novel and potent antitumor agents, as single agents and in combination therapies, to address limitations of current agonistic anti-DR5 antibodies and a promising future approach to overcome tumor-associated resistance to therapeutical apoptosis induction.
Therapeutic strategies that combine rhTRAIL with other anticancer therapeutics showed better clinical responses in patients with advanced cancer compared to mono-treatment of either therapeutics. However, the crucial question here is how to select optimal combinations that provide sufficient antitumor activity to treat patients. Previously, we and others constructed and preclinically evaluated a host of antibody-based TRAIL fusion proteins. Based on the data presented in this thesis, TRAIL fusion proteins can now be divided into three subgroups, 1) tumor-targeted TRAIL fusion proteins, such as anti-EGFR:TRAIL and anti-MCSP:TRAIL, and 2) immune cells-arming TRAIL fusion proteins, such as anti-CD3:TRAIL and anti-CD7:TRAIL, and TRAIL fusion proteins that blocks immune suppressive signal pathways. Tumor microenvironment has been recognized as an essential contributor for cancer progression and drug resistance. In Chapter 4 and 5 we demonstrated that TRAIL fusion proteins may be exploited to counteract the immunosuppressive tumor microenvironment and to reactivate functionally impaired immune effector cells. This new class of TRAIL fusion proteins may present a promising approach in cancer immunotherapy.

The risk of off-target toxicity by TNFR agonist remains an essential hurdle for their clinical use. In Chapter 6 we addressed this issue by developing a novel pretargeting strategy using recombinant fusion proteins in which a soluble form of TRAIL, FasL or CD40L is genetically fused to a high-affinity anti-fluorescein scFv antibody fragment (scFvFITC). Instead of engineering a different scFv:TNFSF fusion protein for each unique tumor antigen, scFvFITC:TNFSF fusion can be used in combination with any available FITC-labeled antibody, either directed to cancer cells or immune effector cells. Importantly, the activity of these scFvFITC:TNFSF fusion proteins can be inhibited by applying nontoxic fluorescein, allowing the possibility to carefully titrate their activity and/or attenuate on target/off tumor toxicity. Recently, a comparable approach for remote control using fluorescein has been exploited to regulate the activity of CAR T-cells. Possibilities to remotely attenuate or even neutralize drug activity have great promise for cancer immunotherapy.
References