Perspective

Targeting death receptors: is this trail still hot?

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Despite tremendous improvements in the survival of patients with pediatric solid tumors, outcomes for metastatic solid tumors and tumors that fail upfront therapy remains dismal (1,2). Further dose intensification of cytotoxic chemotherapy and radiation has not improved survival and new treatment strategies are desperately needed. Over the last few decades several newer biological agents, which target specific mutations or pathways responsible cancer growth have shown promise in pre-clinical testing (3-5). Many of them are already in clinical trials as a single agent and/or in combination with cytotoxic chemotherapy with mixed results (6,7). In addition, last decade has seen an increasing understanding of apoptosis defects observed in many cancer cell types and its role in promoting relentless cancer cell growth and drug resistance (8). This has generated a great deal of interest in the development of therapeutics to activate cancer cell-death-pathway with the ultimate goal of restoring self-destruction by cancer cells.

Two different pathways initiate Apoptosis: the death receptors-mediated extrinsic pathway and the mitochondria-involved intrinsic pathway (9). Death receptors (DR1-DR6) belong to the tumor necrosis factor receptor (TNFR) family that can engage intracellular apoptotic pathways upon binding of their cognate ligands of the tumor necrosis factor (TNF) family. TRAIL (also known as Apo2L) is a TNF family ligand (10) that binds on the death receptors, DR4 (also known as TRAIL-R1) (11,12) and DR5 (also known as TRAIL-R2) (13) and activates apoptotic pathways selectively in cancer cells.

While the molecular basis for this selectivity by TRAIL is poorly understood, it has certainly made TRAIL pathway an attractive target for cancer treatment, Higher expression of the TRAIL- receptors in tumor cells, relative high level of decoy receptors in normal cells (11), and non-functionality of the pathway at more downstream levels can potentially explain the mechanism (13,14). TRAIL targeting strategies can be divided in two broad categories: recombinant human TRAIL (rh-TRAIL) generated mainly from the extracellular domain (Untagged and tagged) (15) and monoclonal antibodies (MAbs) against DR4 and DR5 (humanized mouse MAb and fully human MAbs) (16-18). MAbs have proven to be effective clinical cancer therapeutics because they can selectively target specific antigens and have a much longer half-life than rh-TRAIL ligands (19,20). Both rh-TRAIL and MAbs have shown excellent activity in pre-clinical studies against adult as well as pediatric tumor models including breast cancer, osteosarcoma, Ewing's sarcoma and rhabdomyosarcoma (21-23). They have also shown synergy with cytotoxic chemotherapy and radiation in pre-clinical models (24-27). A number of fully human DR4 and DR5 MAbs are now in Phase-I-II clinical trials in adults including HGS-ETR1 (mapatumumab) (20,28) and HGS-ETR2 (lexatumumab) (19,29).

In the Nov 2012 issue of *Journal of Clinical Oncology* (*J Clin Oncol 30:4141-7*) Merchant MS and colleagues have published results of the first Pediatric Phase-I Trial and Pharmacokinetic Study of Lexatumumab in Patients with Solid Tumors (30). I want to congratulate the authors on a well designed and nicely executed study. Primary findings of this study were very similar to two other adult Phase-I studies done with this agent previously (19,29). Lexatumumab was tolerated very well in children with relapsed solid tumors, when administered once every 14 days. Despite the fact that pediatric patients in this trial were heavily pre-treated, only one patient experienced DLT, Patients tolerated adult maximum tolerated dose

(MTD) of 10 mg/kg every 2 weeks relatively well. In particular, liver and gastrointestinal toxicities seen in pre-clinical models and adult trials, were minimal in this study and no cumulative toxicities were seen in a patient treated for almost 2 years. Pharmacokinetic studies showed a profile similar to that seen in adult studies, with linear increase in drug exposure from dose of 5-10 mg/kg dose levels and adult equivalent accumulation indices validating every 14 days dosing. Overall area under the curve (AUC) was lower in children but was within 1 standard deviation of adult exposure. Mirroring the adult Phase-I trial experience, the agent was found not to be very immunogenic and no antibodies were detected against Lexatumumab (19,29).

Twenty one out of twenty four patients had either soft tissue or bone sarcoma, a disease group most likely to show clinical response based on pre-clinical studies and adult trials. The pediatric trial did show hints of activity although no objective responses were seen. A patient with osteosarcoma showed prolonged improvement in clinical symptoms and disappearance of FDG activity, a patient with Ewing's sarcoma showed mixed response with disappearance of mass which was irradiated before trial enrollment and a patient with hepatoblastoma showed a dramatic reduction in tumor marker. Correlative studies showed strong staining for TRAIL-R2 in 7/14 of tissue slides and 7/9 had strong caspase-8 staining while none stained strongly for TRAIL-R1. There was no correlation between clinical benefit and strength of TRAIL-R2 or Caspase-8 staining, a finding also seen in adult trials (19,29). However, in this pediatric trial there was a correlation noted between no TRAIL-R2 staining and rapid progression on the study, with all 4 patients with no staining progressing within first 2 cycles.

This pediatric Phase-I trial of Lexatumumab shows that it is an agent, which is nicely tolerated in children and shows a hint of clinical activity as a single agent in a Phase-I clinical setting. Authors made several important observations and raise many intriguing questions as to how best to move forward with this agent in pediatric oncology. First, the trial confirms findings of the two previous adult Phase-I trials, that soft tissue and bone sarcoma patients seem to show some benefit and merits further testing in a Phase-II setting (19,29,30). Second, what is the role of prior radiation therapy in patients receiving lexatumumab? Two out of three cases showing some clinical benefit on a trial had received radiation 4 weeks prior to trial enrolment. This observation was also supported by pre-clinical studies, which suggests that radiation therapy up-regulates TRAIL-R2 expression in tumor tissue without increasing the toxicity in normal tissues (27,31). Third, this pediatric study also showed correlation between lack of TRAIL-R2 expression and rapid progression on trial. However, numbers are very small and majority of patients had their tissue collected at the time of original diagnosis, so the true TRAIL-R1, TRAIL-R2 and caspase-8 expression at the time of enrolment is unknown. This is contrary to majority of available evidence, which suggests that death receptor and caspases expression are not predictive of clinical response (19,29). There is some evidence that O-Glycosyltransferase expression is required for DR4/DR5 clustering and Caspase-8 activation and its levels are predictive of sensitivity to rh-TRAIL in a large number of cancer cell lines (32), but this needs to be confirmed in patient samples in a clinical trial.

So, where do we go from here? There are several questions, which needs to be answered before we move forward. What's the most likely patient population who might benefit? What's the best modality for combination therapy? Is it Radiation and/or chemotherapy or with new biologic agents? Based on early clinical experience in pediatrics, testing of lexatumumab in pediatric sarcomas with either sequential or concurrent radiation therapy seems most logical. In addition, several chemotherapeutic agents have also shown ability to modulate the TRAIL receptor expression in pre-clinical settings by inducing DR4/DR5 and Fas expression in human cancer cells (25,33-35) or downregulated c-FLIP (36). Chemotherapy agents most likely to potentiate the effect of TRAIL agonists include etoposide, cytarabine, doxorubicin, cisplatin, methotrexate and bleomycin, which are some of the most commonly used chemotherapeutic agents in pediatric oncology. Also, there is evidence to suggest that when TRAIL therapy is combined with either radiation therapy or chemotherapy, it can overcome resistance to any of these modalities as single agent by upregulating death receptors DR4 or DR5, caspase-3, caspase-8, or bax, or through downregulating Bcl-XL or cFLIP (37). This would have implications in using TRAIL combination therapy in tumors where either of those modalities were known to be ineffective in the past. These finding argues strongly in favor of combination trial between TRAIL receptor agonists and chemotherapy and/or radiation therapy and there are several such Phase-II trials ongoing in adults with solid tumors as well with hematological malignancies (28).

Finally, in addition to chemotherapy, there are several new biologics including HDAC inhibitors and Proteasome

inhibitors, which are also known to increase the expression of TRAIL receptors DR5 and/or DR4, reduce the levels of c-FLIP and enhance TRAIL induced apoptosis in both hematopoietic and/or solid tumor models (38-42). A combination of these biologics and TRAIL agonists are in clinical trials in adults with hematological malignancies and solid tumors and certainly merits further testing against pediatric tumors.

In summary, lexatumumab and other TRAIL agonists are very exciting new class of drugs with very favorable toxicity profile, easy schedule of administration and hints of clinical activity in early phase pediatric clinical trials. Their true place in the new treatment paradigm is yet to be defined and their ultimate success will depend on how quickly we can answer some of these remaining questions.

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Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

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