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Probing the ligand receptor interface of TNF ligand family members RANKL and TRAIL

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Chapter 1

General introduction and scope of the thesis

TNF superfamily

The discovery of tumor necrosis factor (TNF) superfamily dates back to the middle of the nineteenth century when O'Malley found a tumor-necrotizing factor in the serum that could mediate tumor regression effects [1]. Carswell's group renamed this factor as tumor necrosis factor and reported that macrophages were the source of this factor [2]. In 1968, Granger and colleagues described another factor called lymphotoxin (LT) produced by lymphocytes, which could also kill tumor cells [3]. Until 1984, Aggarwal's group determined and compared the amino acid sequence of TNF and LT, indicating that the two proteins were homologous [4]. Therefore, they were renamed to TNF- α and TNF- β , respectively. These two factors laid the foundation for the identification of the TNF superfamily.

At present, in total 19 TNF superfamily ligands and 29 receptors have been identified (shown in Table. 1). The TNF superfamily ligands are type II trimeric transmembrane proteins with a C-terminal TNF homology domain (THD). THD is a sequence of around 150 amino acids, which contains a conserved framework of aromatic and hydrophobic residues to fold and form trimeric proteins [5]. The individual monomers are β -sandwich structures containing β strands and loops that form a "jelly-roll" structure: the inner sheets are responsible for trimerization and the outer sheets are for receptor binding [5,6]. The TNF superfamily receptors are type I or type III membrane or soluble proteins containing cysteine-rich domains (CRD) for ligand binding [7].

Among the large family of TNF ligands and receptors, there are 19 ligands and 29 different receptors, indicating the diversity of ligand-receptor interactions. As shown in Table. 1, TNF ligands can bind to one specific or multiple receptors, and different ligands can bind to the same receptor. The TNF receptors can be divided into 3 groups according to their cytoplasmic sequences and their cellular signal pathways [8]. The first group receptors, including Fas, TNFR1, DR3, DR4, DR5, and DR6, contain a death domain (DD) in the cytoplasmic tail. Activation of these DD-containing receptors can lead to the recruitment of adaptor proteins such as TNFR associated death domain (TRADD) or Fas-associated death domain (FADD), which on their turn can activate the caspase cascade and finally induce apoptosis [9]. The second group, including TNFR2, CD40, CD30, CD27, LT β R, OX40, 4-1BB, BAFFR, BCMA, TACI, RANK, NGFR, HVEM, GITR, TROY, EDAR, XEDAR, RELT, and Fn14, contains TNFR associated factor (TRAF)-interacting motifs (TIM). Activation of these TIM-containing receptors can lead to the activation of downstream signaling pathways, including

nuclear factor- κ B (NF- κ B), mitogen-associated protein kinases (MAPK) such as p38, c-JNK, and the extracellular signal-regulated kinases (ERK) as well as phosphoinositide 3-kinase (PI3K) and Akt [8,10], which finally induce cell survival and inflammation. The third group of TNF receptors includes Decoy receptor 1 (DcR1), DcR2, DcR3, and osteoprotegerin (OPG), which do not contain functional intracellular signaling domains or motifs. They act as decoy receptors to compete and block the binding between the ligand and other functional receptors [8,11,12].

Almost all of the TNF superfamily ligands are expressed by immune cells, while the receptors are expressed by a wide variety of cells [13]. The binding between the TNF superfamily ligands and receptor will cause biological effects such as tumor regression, immune system regulation and hematopoiesis. However, dysregulation of the binding between the ligand and receptor also leads to various diseases [13]. In this thesis, we mainly focus on two TNF superfamily members, RANKL and TRAIL.

Table 1: Ligands and receptors of TNF superfamily [7]

Ligands		Receptors
Symbol	Common name (alias)	Common name
TNFSF1	TNF- α	TNFR1/TNFR2
TNFSF2	TNF- β (LT- α)	TNFR1/TNFR2/HVEM/ LT β R
TNFSF3	LT- β	LT β R
TNFSF4	OX40L (CD252)	OX40
TNFSF5	CD40L (CD154)	CD40
TNFSF6	FasL (CD95L/Apo1L)	Fas/DcR3
TNFSF7	CD27L (CD70)	CD27
TNFSF8	CD30L (CD153)	CD30
TNFSF9	4-1BBL	4-1BB
TNFSF10	TRAIL (Apo2L)	DR4/DR5/DcR1/DcR2/OPG
TNFSF11	RANKL (OPGL/ODF/TRANCE)	RANK/OPG
TNFSF12	TWEAK (Apo3L)	Fn14
TNFSF13	APRIL (TALL-2/TRDL-1)	TACI/BCMA
TNFSF13B	BAFF (BLYS/THANK)	BAFF-R/TACI/BCMA
TNFSF14	LIGHT (HVEM/LT- γ)	LT β R/DcR3/HVEM
TNFSF15	VEGI (TL1A)	DcR3/DR3
TNFSF18	GITRL	GITR
	EDA-A1	EDAR
	EDA-A2	XEDAR
	N.D.	DR6
	N.D.	RELT
	N.D.	TROY

N. D., not determined.

RANKL/RANK/OPG system

In 1981, Rodan and Martin formulated a detailed hypothesis that osteoblasts influence the formation of osteoclasts [14]. In the late 1990s, OPG was discovered and patented as an important regulator of bone density [15]. Subsequent research was undertaken to identify the ligand of OPG, and contemporaneously an alternative strategy was used to identify TNFR superfamily homologous. As a result, the ligand of receptor activator of nuclear factor κ B (RANKL), also known as OPGL, ODF and TRANCE, and its receptor RANK were discovered by different independent groups [16–18].

The RANKL/RANK/OPG system was first found to play an important role in bone remodeling system [19]. In normal conditions, bone homeostasis is maintained by a balance between old bone resorption by osteoclasts and new bone formation by osteoblasts [20]. RANKL is expressed on osteoblasts and RANK is expressed on osteoclast precursor cells [21]. The interaction between RANKL and RANK can induce the differentiation of osteoclasts from osteoclast precursors, and promote osteoclasts activation and survival [20]. The essential signaling pathway after RANKL stimulation includes the adaptor protein TNFR-associated factors (TRAFs) recruitment, NF- κ B activation and translocation, and osteoclastogenic genes transcription [22]. OPG, which is also produced by osteoblasts, is a soluble decoy receptor and competes with RANK for RANKL binding [23]. Imbalances in the RANK/RANKL/OPG pathway can dysregulate the bone remodeling to either bone formation or resorption, therefore leading to diseases such as osteoporosis or osteopetrosis.

Apart from the role in bone remodeling, RANKL/RANK signaling also plays important roles in other tissues, since they are produced by a variety of cell types and tissues [22]. Recent studies indicate a number of other physiological and pathophysiological processes involving RANKL/RANK pathways like glandular development and lactation, cancer cell proliferation and metastasis and adaptive immunity [24]. More interestingly, this system may also play a role in fibrosis, which is characterized by excessive accumulation of extracellular matrix (ECM) [25]. In bone tissue, RANKL can bind to RANK on osteoclasts and induce bone ECM degradation. However, whether and how this signaling pathway regulates fibrotic tissue ECM degradation is unclear up to now.

Due to the important roles of the RANK-RANKL pathway in bone and other tissues, targeting RANKL or RANK with either agonistic or antagonistic compounds is a promising strategy for therapeutic intervention. Initially, OPG-Fc/variants or RANKL-targeted peptides were

developed as RANKL scavengers to prevent bone loss and inflammation [21,26,27]. Furthermore, denosumab, an FDA-approved RANKL-specific antibody, was developed and used in the treatment of osteoporosis and bone metastases [28]. However, through titrating away RANKL but not the receptor, they all carry the risk of immune reactions. Therefore, strategies with less immune response are still needed. One other approach was to focus on novel structure-based RANKL variants, which might be of interest to target the receptor directly and trigger the autoimmune system with less impact compared to antibody.

TRAIL and its receptors

TNF-related apoptosis inducing ligand (TRAIL), also known as Apo2 ligand (Apo2L), was discovered in 1995 [29,30]. It is an important ligand protein of TNF superfamily that selectively induces apoptosis in a variety of tumor cells [31]. TRAIL interacts with five cognate receptors, namely death receptor 4 (DR4/TRAIL-R1), death receptor 5 (DR5/TRAIL-R2), decoy receptor 1 (DcR1/TRAIL-R3), decoy receptor 2 (DcR2/TRAIL-R4), and the soluble receptor osteoprotegerin (OPG) [32]. Both DR4 and DR5 contain the death domain (DD) in the intracellular part. Therefore, upon binding to DR4 and DR5, TRAIL can activate the extrinsic pathway through the recruitment of the Fas-associated death domain and the activation of caspase-8 [9,33]. The other receptors act as decoys receptors since DcR1 lacks a cytosolic region and DcR2 only contains a truncated, non-functional death domain [34]. OPG is a soluble protein showing weaker affinity to TRAIL compared to other membrane-bound receptors. However, it can still block the induction of apoptosis through sequestering the available TRAIL [35].

TRAIL can initiate the extrinsic apoptosis pathway through binding to DR4 or DR5, which results in the recruitment of caspase-8 and FADD to form the death-inducing signaling complex (DISC) [9]. This can further activate the downstream effector caspase-3, caspase-6, and caspase-7 and result in apoptosis [36]. In some cases, DISC activation is not sufficient to trigger the caspase cascade and the activation of the intrinsic pathway gets involved. Caspase-8 activation cleaves Bid to truncated Bid (tBid), which further interacts with Bax and Bak on the mitochondrial membrane to promote the release of apoptotic factors like cytochrome c. These factors will form the apoptosome through binding to apoptotic peptidase activating factor 1 (Apaf-1) and the initiator caspase-9, which in turn activates caspase-3, caspase-6, and caspase-7 to induce apoptosis [37].

Although TRAIL has been recognized as a representative anti-cancer agent, TRAIL resistance in tumor cells has been considered as a problem for a long time. This resistance may occur at different levels in the signaling pathways, including decoy receptors competition and dysregulation, DISC inhibition, reduced caspase function, imbalance of anti- and pro- proteins expression [35,38]. Therefore, the understanding of the mechanisms of TRAIL resistance and ways to improve TRAIL sensitivity of tumor cells is important for the development of therapeutic approaches with TRAIL.

Recombinant human TRAIL (rhTRAIL, aa 114-281) has been mostly used and developed as a clinical anti-cancer drug [39,40]. Clinical phase I studies showed this rhTRAIL was safe in patients with advanced cancer. However, there was no clinical benefit of rhTRAIL due to its fast kinetics and short half-life. On the other hand, rhTRAIL binds to all five TRAIL receptors, including three decoy receptors, which interfere with TRAIL-induced apoptosis. To overcome TRAIL resistance caused by decoy receptors competition, DR-specific TRAIL variants or antibodies are becoming promising therapeutic approaches in cancer treatment.

Scope of the thesis

The work described in this thesis is aimed to probe the ligand-receptor interface of TNF superfamily members RANKL and TRAIL, and the design and characterization of novel recombinant RANKL and TRAIL variants for their use as potential therapeutics.

Bone, as a dynamic tissue, is maintained by continuous renewal. An imbalance in bone resorption and formation can lead to a range of disorders, such as osteoporosis or osteopetrosis. The RANK/RANKL pathway plays an important role in bone remodeling. In **Chapter 2**, we review the prospective of interfering with the RANK/RANKL pathway as a therapeutic target for bone diseases and discuss the role of the soluble receptor OPG as a therapeutic in bone diseases. Then we focus on the possibility to develop antagonistic and agonistic variants of RANKL based on computational protein design. Finally, we discuss the development of antagonistic RANKL variants by changing the stoichiometry of the RANKL molecule.

In **Chapter 3**, we investigate the effect of mutations at position I248 in the DE-loop of RANKL on the interaction of RANKL with RANK and subsequent osteoclastogenesis activation. Two single mutants, RANKL I248Y and I248K, were found to maintain binding

and have the ability to reduce wild type RANKL-induced osteoclastogenesis. The generation of RANK-antagonists based on RANKL structure is a promising strategy for the exploration of new therapeutic approaches against osteoporosis.

RANKL/RANK/OPG signaling system was found to play an important role in the regulation of ECM formation and degradation in bone tissue. However, whether and how this signaling pathway plays a role in fibrotic tissue ECM degradation is unclear up to now. Interestingly, increased decoy receptor OPG levels are found in fibrotic tissues. In **Chapter 4**, we investigate the role of RANKL/RANK/OPG pathway in fibrosis. We hypothesize that RANKL can stimulate RANK on macrophages and induce the process of ECM degradation. This process may be inhibited by highly expressed OPG in fibrotic conditions. In this case, RANKL mutants that can bind to RANK without binding to OPG might become promising therapeutic candidates. We built a structure-based library containing 44 RANKL mutants and found that RANKL_Q236D can activate RAW cells to initiate the process of ECM degradation and is able to escape from the obstruction by exogenous OPG.

In **Chapter 5**, to achieve a delivery system for targeting RANKL_Q236D to the fibrotic tissues, without influencing the bone remodeling, we constructed replication-deficient adenoviruses Ad-RANKL WT and Ad-RANKL Q236D. The functionality of virally produced RANKL_WT and RANKL_Q236D was confirmed, and it turned out that virally produced RANKL_Q236D can activate RAW 264.7 cells and make them escape from the inhibition by exogenous OPG. The generation of Ad-RANKL Q236D is a powerful tool that might lead to new therapies against fibrosis.

Since OPG also acts as the decoy receptor of TRAIL, we are also interested in the ligand-receptor interface of TRAIL and its receptors, and TRAIL resistance caused by its decoy receptors. In **Chapter 6**, we compare the sensitivity of TRAIL in senescent and proliferating cancer cells. We found that therapy-induced senescence resulted in an increased expression of the pro-apoptotic TRAIL receptor death receptor 5 (DR5), as well as an increase in TRAIL decoy receptors DcR1, DcR2, and soluble decoy receptor OPG. A DR5 selective TRAIL variant (DHER), which is unable of binding to OPG, DcR1 or DR4, is more effective in inducing apoptosis of senescent breast cancer cells compared to wild-type TRAIL.

High expression of OPG is also found in breast tumor cells, which inhibits TRAIL-induced apoptosis. **Chapter 7** reports on a structure-based design strategy to obtain TRAIL variants with decreased affinity to OPG. We found the double mutant TRAIL_D269H/Y209M and the

triple mutant TRAIL_D269H/Y209M/K179P showed lowered binding to OPG compared to TRAIL_DHER, and these variants can induce apoptosis in breast cancer cells MDA-MB-231 and MDA-MB-436 in the presence of OPG.

In **Chapter 8**, we summarize the work presented in this thesis and describe some suggestions for future perspectives.

References:

- 1 O'Malley WE, Achinstein B & Shear MJ (1962) Action of bacterial polysaccharide on tumors. ii. damage of sarcoma 37 by serum of mice treated with serratia marcescens polysaccharide, and induced tolerance. *J. Natl. Cancer Inst.* **29**, 1169–1175.
- 2 Carswell EA, Old LJ, Kassel RL, Green S, Fiore N & Williamson B (1975) An endotoxin induced serum factor that causes necrosis of tumors. *Proc. Natl. Acad. Sci. U. S. A.* **72**, 3666–3670.
- 3 Ezperimental T (1968) Lymphocyte in vitro Cytotoxicity: Lymphotoxins of Several Mammalian Species Relationship between Cell Size and Time of Initiation of DNA Replication. **219**, 1967–1968.
- 4 Aggarwal BB, Kohr WJ, Hass PE, Moffat B, Spencer SA, Henzel WJ, Bringman TS, Nedwin GE, Goeddel D V. & Harkins RN (1985) Human tumor necrosis factor: Production, purification, and characterization. *J. Biol. Chem.* **260**, 2345–2354.
- 5 Bodmer JL, Schneider P & Tschopp J (2002) The molecular architecture of the TNF superfamily. *Trends Biochem. Sci.* **27**, 19–26.
- 6 Nelson CA, Warren JT, Wang MW-H, Teitelbaum SL & Fremont DH (2012) RANKL employs distinct binding modes to engage RANK and the osteoprotegerin decoy receptor. *Structure* **20**, 1971–1982.
- 7 Ware CF (2003) Introduction: The TNF superfamily. *Cytokine Growth Factor Rev.* **14**, 181–184.
- 8 Dempsey PW, Doyle SE, He JQ & Cheng G (2003) The signaling adaptors and pathways activated by TNF superfamily. *Cytokine Growth Factor Rev.* **14**, 193–209.
- 9 Kischkel FC, Lawrence DA, Chuntharapai A, Schow P, Kim KJ & Ashkenazi A (2000) Apo2L/TRAIL-Dependent Recruitment of Endogenous FADD and Caspase-8 to Death Receptors 4 and 5. *Immunity* **12**, 611–620.
- 10 Boyle WJ, Simonet WS & Lacey DL (2003) Osteoclast differentiation and activation. *Nature* **423**, 337–342.
- 11 Nagy V & Penninger JM (2015) The RANKL-RANK Story. *Gerontology* **61**, 534–542.

- 12 Marsters SA, Sheridan JP, Pitti RM, Huang A, Skubatch M, Baldwin D, Yuan J, Gurney A, Goddard AD, Godowski P & Ashkenazi A (1997) A novel receptor for Apo2L/TRAIL contains a truncated death domain. *Curr. Biol.* **7**, 1003–1006.
- 13 Aggarwal BB (2003) Signalling pathways of the TNF superfamily: A double-edged sword. *Nat. Rev. Immunol.* **3**, 745–756.
- 14 Rodan GA & Martin TJ (1982) Role of osteoblasts in hormonal control of bone resorption - a hypothesis. *Calcif. Tissue Int.* **34**, 311.
- 15 Simonet W., Lacey D., Dunstan C., Kelley M, Chang M-S, Lüthy R, Nguyen H., Wooden S, Bennett L, Boone T, Shimamoto G, DeRose M, Elliott R, Colombero A, Tan H-L, Trail G, Sullivan J, Davy E, Bucay N, Renshaw-Gegg L, Hughes T., Hill D, Pattison W, Campbell P, Sander S, Van G, Tarpley J, Derby P, Lee R & Boyle W. (1997) Osteoprotegerin: A Novel Secreted Protein Involved in the Regulation of Bone Density. *Cell* **89**, 309–319.
- 16 Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki SI, Tomoyasu A, Yano K, Goto M, Murakami A, Tsuda E, Morinaga T, Higashio K, Udagawa N, Takahashi N & Suda T (1998) Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. *Proc. Natl. Acad. Sci. U. S. A.* **95**, 3597–3602.
- 17 Anderson DM, Maraskovsky E, Billingsley WL, Dougall WC, Tometsko ME, Roux ER, Teepe MC, DuBose RF, Cosman D & Galibert L (1997) A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. *Nature* **390**, 175–179.
- 18 Wong BR, Rho J, Arron J, Robinson E, Orlinick J, Chao M, Kalachikov S, Cayani E, Bartlett FS, Frankel WN, Lee SY & Choi Y (1997) TRANCE is a novel ligand of the tumor necrosis factor receptor family that activates c-Jun N-terminal kinase in T cells. *J. Biol. Chem.* **272**, 25190–25194.
- 19 Trouvin AP & Gøtz V (2010) Receptor activator of nuclear factor- κ B ligand and osteoprotegerin: maintaining the balance to prevent bone loss. *Clin. Interv. Aging* **5**, 345–354.
- 20 Tanaka S, Nakamura K, Takahashi N & Suda T (2005) Role of RANKL in physiological and pathological bone resorption and therapeutics targeting the RANKL-RANK signaling system. *Immunol. Rev.* **208**, 30–49.
- 21 Naidu VGM, Dinesh Babu KR, Thwin MM, Satish RL, Kumar P V & Gopalakrishnakone P (2013) RANKL targeted peptides inhibit osteoclastogenesis and attenuate adjuvant induced arthritis by inhibiting NF- κ B activation and down regulating inflammatory cytokines. *Chem. Biol. Interact.* **203**, 467–479.
- 22 Boyce BF & Xing L (2007) Biology of RANK, RANKL, and osteoprotegerin. *Arthritis Res. Ther.* **9**.
- 23 Rachner TD, Khosla S & Hofbauer LC (2011) Osteoporosis: now and the future. *Lancet* **377**, 1276–1287.

- 24 Sisay M, Mengistu G & Edessa D (2017) The RANK/RANKL/OPG system in tumorigenesis and metastasis of cancer stem cell: Potential targets for anticancer therapy. *Onco. Targets. Ther.* **10**, 3801–3810.
- 25 Adhyatmika A, Putri KSS, Beljaars L & Melgert BN (2015) The elusive antifibrotic macrophage. *Front. Med.* **2**, 1–11.
- 26 Body JJ, Greipp P, Coleman RE, Facon T, Geurs F, Ferman JP, Harousseau JL, Lipton A, Mariette X, Williams CD, Nakanishi A, Holloway D, Martin SW, Dunstan CR & Bekker PJ (2003) A phase I study of AMG-0007, a recombinant osteoprotegerin construct, in patients with multiple myeloma or breast carcinoma related bone metastases. *Cancer* **97**, 887–92.
- 27 Higgs JT, Jarboe JS, Lee JH, Chanda D, Lee CM, Deivanayagam C & Ponnazhagan S (2015) Variants of osteoprotegerin lacking TRAIL binding for therapeutic bone remodeling in osteolytic malignancies. *Mol. cancer Res.* **33**, 395–401.
- 28 Ta HM, Nguyen GTT, Jin HM, Choi J, Park H, Kim N, Hwang H-Y & Kim KK (2010) Structure-based development of a receptor activator of nuclear factor- κ B ligand (RANKL) inhibitor peptide and molecular basis for osteopetrosis. *Proc. Natl. Acad. Sci.* **107**, 20281–20286.
- 29 Ashkenazi A, Pitti R, Marsters S, Ruppert S, Donahue C & Moore A (1996) Induction of apoptosis by APO-2 ligand, a new member of the TNF cytokine family. *FASEB J.* **10**.
- 30 Wiley SR, Schooley K, Smolak PJ, Din WS, Huang CP, Nicholl JK, Sutherland GR, Smith TD, Rauch C, Smith CA & Goodwin RG (1995) Identification and characterization of a new member of the TNF family that induces apoptosis. *Immunity* **3**, 673–682.
- 31 Van Roosmalen IAM, Quax WJ & Kruyt FAE (2014) Two death-inducing human TRAIL receptors to target in cancer: Similar or distinct regulation and function? *Biochem. Pharmacol.* **91**, 447–456.
- 32 Van der Sloot AM, Tur V, Szegezdi E, Mullally MM, Cool RH, Samali A, Serrano L & Quax WJ (2006) Designed tumor necrosis factor-related apoptosis-inducing ligand variants initiating apoptosis exclusively via the DR5 receptor. *Proc. Natl. Acad. Sci. U. S. A.* **103**, 8634–8639.
- 33 Bodmer J-L, Holler N, Reynard S, Vinciguerra P, Schneider P, Juo P, Blenis J & Tschopp J (2000) TRAIL receptor-2 signals apoptosis through FADD and caspase-8. *Nat. Cell Biol.* **2**, 241–243.
- 34 Reis CR, Van der Sloot AM, Szegezdi E, Natoni A, Tur V, Cool RH, Samali A, Serrano L & Quax WJ (2009) Enhancement of antitumor properties of rhTRAIL by affinity increase toward its death receptors. *Biochemistry* **48**, 2180–2191.
- 35 Kretz AL, Trauzold A, Hillenbrand A, Knippschild U, Henne-Bruns D, von Karstedt S & Lemke J (2019) Trailblazing strategies for cancer treatment. *Cancers (Basel)*. **11**, 1–30.
- 36 Yuan X, Gajan A, Chu Q, Xiong H, Wu K & Wu GS (2018) Developing TRAIL/TRAIL death receptor-based cancer therapies. *Cancer Metastasis Rev.* **37**, 733–748.

- 37 Mahalingam D, N.A.M. Oldenhuis C, Szegezdi E, J. Giles F, G.E. de Vries E, de Jong S & T. Nawrocki S (2011) Targeting Trail Towards the Clinic. *Curr. Drug Targets* **12**, 2079–2090.
- 38 Wong SHM, Kong WY, Fang CM, Loh HS, Chuah LH, Abdullah S & Ngai SC (2019) The TRAIL to cancer therapy: Hindrances and potential solutions. *Crit. Rev. Oncol. Hematol.* **143**, 81–94.
- 39 Yu R, Albarenque SM, Cool RH, Quax WJ, Mohr A & Zwacka RM (2014) DR4 specific TRAIL variants are more efficacious than wild-type TRAIL in pancreatic cancer. *Cancer Biol. Ther.* **15**, 1658–1666.
- 40 Herbst RS, Eckhardt SG, Kurzrock R, Ebbinghaus S, O’Dwyer PJ, Gordon MS, Novotny W, Goldwasser MA, Tohnya TM, Lum BL, Ashkenazi A, Jubb AM & Mendelson DS (2010) Phase I dose-escalation study of recombinant human Apo2L/TRAIL, a dual proapoptotic receptor agonist, in patients with advanced cancer. *J. Clin. Oncol.* **28**, 2839–2846.

