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Targeted therapy, molecular imaging and biomarkers in cancer treatment

den Hollander, Martha Willemine

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Chapter 2

Translating TRAIL-receptor targeting agents to the clinic

M.W. den Hollander, J.A. Gietema, S. de Jong, A.M.E. Walenkamp, A.K.L. Reyners,
C.N.A.M. Oldenhuis, E.G.E. de Vries

Department of Medical Oncology, University of Groningen, University Medical Center
Groningen, Groningen, The Netherlands

ABSTRACT

The extrinsic apoptotic pathway can be activated by the endogenous ligand TRAIL (Tumor Necrosis Factor (TNF)-Related Apoptosis-Inducing Ligand) by binding to the death receptors TRAIL-R1 and TRAIL-R2 on the cell surface. This pathway is currently evaluated as an anticancer treatment strategy. Both recombinant human TRAIL and several agonistic antibodies against TRAIL-R1 and R2 have been studied in single agent and combination studies and proved to be safe and well tolerated. In this article, the clinical studies published to date will be reviewed. Also, future perspectives and biomarker studies for selecting patients that will benefit from these agents will be discussed.

INTRODUCTION

There are several different ways of inducing apoptosis in malignant cells as an anticancer treatment strategy. The most frequently exploited ways of treating tumors is to induce DNA damage via chemotherapy and/or radiotherapy, thereby activating the mitochondrial (intrinsic) apoptotic pathway. An important regulator of the intrinsic apoptotic pathway is the tumor suppressor p53, which can induce apoptosis in response to DNA damage inflicted by chemotherapy and radiation. However, p53 function in tumor cells is often lost, resulting in resistance to chemotherapy. In addition, chemotherapy does not selectively affect tumor cells, but also induces damage to normal cells.

Another way of inducing cell death is by stimulation of apoptosis via the extrinsic pathway. The extrinsic pathway is independent of p53 and can be activated by the endogenous ligand TRAIL (Tumor Necrosis Factor (TNF)-Related Apoptosis-Inducing Ligand), a transmembrane protein and a member of the TNF super family [1]. Physiologically, TRAIL is considered to have an anti-inflammatory effect and to play a role in autoimmunity and anti-tumor surveillance [2-4]. TRAIL can induce apoptosis in tumor cells by binding to the death receptors TRAIL-R1 (DR4) and TRAIL-R2 (DR5) on the cell surface. These death receptors are present in a broad range of both normal cells and tumor cells [5]. Interestingly, recombinant human (rh)TRAIL induces cell death only in tumor cells and not in normal cells [6]. What causes this difference is still not elucidated. When death receptors are activated by TRAIL, these receptors undergo homo-trimerization. This trimer forms the death-inducing signaling complex (DISC) together with the Fas-associated death-domain (FADD) and pro-caspases 8 and 10. The activated caspases then activate caspases 3, 6 and 7, eventually resulting in apoptosis. Active caspase 8 also cleaves Bcl-2 interacting domain (Bid) into truncated Bid (tBid), which then triggers the intrinsic apoptotic pathway by activation of caspase 9 and finally caspase 3. Important cellular proteins that inhibit activation of the extrinsic apoptotic pathway are cFLIP, a competitor of caspase 8, and the inhibitor-of-apoptosis proteins (IAPs) that inhibit caspase activity [7]. In preclinical studies, not only rhTRAIL but also the agonistic antibodies against TRAIL-R1 and TRAIL-R2 induced apoptosis in various tumor cell lines, while normal cells were spared [6,8,9]. RhTRAIL and the TRAIL-R antibodies, also called PARAs (pro-apoptotic receptor agonists), in addition enhance the cytotoxic effect of "classic" chemotherapy, targeted therapies and radiotherapy [10-12]. This has led to several studies that are finalized or are ongoing with PARAs as single agent or combined with chemotherapeutic as well as targeted agents. In this review the results of these studies will be summarized and future perspectives and the possible use of biomarkers for selecting eligible patients will be discussed.

CLINICAL STUDIES

In recent years, several phase 1 and 2 single agent and combination studies have been conducted with recombinant human TRAIL (dulanermin, (Amgen/Genentech)) targeting both TRAIL-R1 and TRAIL-R2 and the agonistic monoclonal antibodies to either TRAIL-R1 (mapatumumab (Human Genome Sciences)) or TRAIL-R2 (lexatumumab (Human Genome Sciences), conatumumab (Amgen), drozitumab (Genentech), tigatuzumab (Daiichi-Sankyo) and LBY135

(Novartis)). These trials are summarized in Table 1.

Mapatumumab, lexatumumab, conatumumab and drozitumab are fully human IgG1 antibodies, whereas tigatuzumab is a humanized IgG1 antibody and LBY135 a chimeric (mouse/human) IgG1 antibody.

SINGLE AGENT STUDIES

Dulanermin

In a phase 1 study with dulanermin, 71 patients with advanced cancer received up to 30 mg/kg/day intravenously (iv) for 5 days every 3 weeks. This regime was found to be safe and well tolerated. Two patients with chondrosarcoma achieved a partial tumor response, and were still on treatment after 2.7 and 4.3 years, respectively. Furthermore, in two other sarcoma patients, tumor necrosis was found during surgery after the first cycle of dulanermin therapy, which is possibly an indication of dulanermin induced cell death. The serum half life of dulanermin was found to be 0.5–1 h. No antibodies against dulanermin were detected [13].

Mapatumumab

In two phase 1 trials with mapatumumab up to 10 mg/kg iv every 2 weeks or up to 20 mg/kg iv every 4 weeks, the best responses were stable disease in respectively 19 out of 49 and 12 out of 41 patients with advanced solid tumors. The maximum tolerated doses were not reached [14,15].

In a phase 2 study, patients with colorectal cancer received mapatumumab 10 mg/kg after 2 loading doses of 20 mg/kg iv every 14 days. In a phase 2 study in patients with non-small cell lung cancer (NSCLC), patients received mapatumumab 10 mg/kg every 21 days. In both studies, no objective responses were observed, but respectively 12 out of 38 and 9 out of 32 heavily pretreated patients achieved stable disease [16,17].

In a phase 1b/2 trial in 40 patients with non-Hodgkin's lymphoma (NHL) treated with doses of 3 or 10 mg/kg mapatumumab iv every 21 days, 2 complete responses and 1 partial response were seen in patients with follicular lymphoma and 11 patients achieved stable disease [18].

No anti-mapatumumab antibodies were found in the phase 1 trials. The mean plasma half life value for mapatumumab was found to be 19, 22 and 26 days respectively [14,15,18].

Agonistic TRAIL-R2 antibodies

Lexatumumab was studied as a single agent in two phase 1 studies in patients with advanced solid tumors. The maximum tolerated dose was found to be 10 mg/kg and this dose could be administered every 2 weeks. Dose limiting toxicities, seen in five patients in these 2 studies, consisted of elevations of serum amylase, bilirubin and transaminases. One of these patients developed septicemia and acute renal failure and died 25 days after the lexatumumab administration. Stable disease was achieved in respectively 12 out of 37 and 9 out of 27 patients, while one mixed response was seen in a patient with Hodgkin's lymphoma. In this patient a lung lesion became smaller but other lesions increased in size [19,20]. A phase 1 study with lexatumumab

Table 1: Overview of clinical studies with pro-apoptotic receptor agonists.

Author	Drug	Phase	Tumor type	No. of patients	Combined with	Best response	Results phase 2 randomized studies
Belada 2010 [34]	Dulanermin	2 r.	NHL	48	Arm 1: rituximab + dulanermin Arm 2: rituximab		Arm 1/2: ORR: 61.5%/63.6% CR: 4 (1 unconfirmed)/5 PR: 12/9
Fanale 2008 [33]	Dulanermin	1b	Relapsed low grade NHL	12	Rituximab	3 CR, 3 PR	
Herbst 2010 [13]	Dulanermin	1	Advanced cancer	71	-	2 PR, 31 SD Med. PFS 2.3 mo	
Soria 2010 [31]	Dulanermin	1b	Advanced non squamous NSCLC	24	Paclitaxel, carboplatin, bevacizumab	1 CR, 13 PR ORR 58% Med. PFS 7.2 mo	
Soria 2011 [32]	Dulanermin	2 r.	NSCLC	213	Arm 1: paclitaxel (P)+ carbo- platin (C) Arm 2: P+C + dulanermin (D) (8 mg/kg/5 days) Arm 3: PC + bevacizumab (B) Arm 4: PCB + D (8 mg/kg/5 days) Arm 5: PCB + D (20 mg/kg/2 days)	Arm 1/2/3/4/5 ORR: 39%/38%/50%/40%/40% PFS: 6.1/5.5/7.3/8.6/9.5 OS: 10.1/9.8/15.1/13.9/14.3	
Yee 2009 [35]	Dulanermin	1b	Metastatic colorectal cancer	35	Irinotecan, cetuximab or FOLFIRI	NR	
Greco 2008 [17]	Mapatumumab	2	Advanced NSCLC	32	-	9 SD Med PFS 1.2 mo	
Hotte 2008 [15]	Mapatumumab	1	Advanced solid tumors	41	-	12 SD med PFS 1.7 mo	
Leong 2009 [37]	Mapatumumab	1	Advanced solid tumors	27	Paclitaxel, carboplatin	5 PR 12 SD	

Table 1: continued.

Author	Drug	Phase	Tumor type	No. of patients	Combined with	Best response	Results phase 2 randomized studies
Mom 2009 [36]	Mapatumumab	1	Advanced solid tumors	49	Gemcitabine cisplatin	12 PR 25 SD	
Tolcher 2007 [14]	Mapatumumab	1	Advanced solid tumors	49	-	19 SD	
Trarbach 2010 [16]	Mapatumumab	2	Refractory colorectal cancer	38	-	12 SD med PFS 1.2 mo	
Von Pawel 2010 [38]	Mapatumumab	2 r.	NSCLC	111	Arm 1: paclitaxel + carboplatin (PC) Arm 2: PC + mapatumumab 10 mg/kg Arm 3: PC + mapatumumab 30 mg/kg		Arm 1/2/3 ORR: 30.6%/13.5 %/ 36.1% Med PFS: 4.6/4.6/4.9 Med OS: 10.5/13.6/10.6
Younes 2010 [18]	Mapatumumab	2	Relapsed/refractory NHL	40	-	2CR, 1 PR 11 SD	
Sun 2011 [39]	Mapatumumab	1b	Advanced hepatocellular carcinoma	19	Sorafenib	2 PR, 4 SD	
Merchant 2010 [21]	Lexatumumab	1	Advanced solid tumors (pediatric patients)	24	-	5 SD	
Plummer 2007 [19]	Lexatumumab	1	Advanced solid tumors	37	-	12 SD	
Sikic 2007 [40]	Lexatumumab	1b	Wide range of cancer types	41	Gemcitabine, pemetrexed, doxorubicin, or FOLFIRI	3 PR	
Wakelee 2010 [20]	Lexatumumab	1	Advanced solid tumors	31	-	9 SD 1 mixed response	
Baron 2011 [44]	Droxitumab	1b	Metastatic colorectal cancer	20	Cetuximab + irinotecan or FOLFIRI±bevacizumab	3 PR(1 unconfirmed), 13 SD	

Author	Drug	Phase	Tumor type	No. of patients	Combined with	Best response	Results phase 2 randomized studies
Camidge 2010 [22]	Drozitumab	1	Advanced solid tumors or NHL	50	-	20 SD 3 minor responses	
Karapetis 2010 [41]	Drozitumab	2 r.	NSCLC	62	Arm 1: PCB + Drozitumab Arm 2: PCB + placebo		Arm 1/2: Med PFS: 7.9/7.0 Med OS: 9.9/12.6 ORR: 40%/42%
Rocha Lima 2011 [43]	Drozitumab	1b	Metastatic colorectal cancer	9	FOLFOX + bevacizumab	5 PR (3 unconfirmed), 3 SD	
Wittebol 2010 [42]	Drozitumab	2	NHL	40	Rituximab	2 CR, 18 PR	
Chawla 2010 [49]	Conatumumab	1	Advanced solid tumors	9	AMG 479	3 SD	
Demetri 2012 [51]	Conatumumab	1/2 r.	Metastatic/unresectable soft tissue sarcoma	134	Arm 1: conatumumab + doxorubicin Arm 2: placebo + doxorubicin		Arm 1/2: Med PFS: 5.6/6.8 Med OS: 18.2/21.6
Doi 2011 [25]	Conatumumab	1	Advanced solid tumors	18		9 SD	
Herbst 2010 [24]	Conatumumab	1	Advanced solid tumors	37		1 PR, 14 SD	
Kindler 2009 [47]	Conatumumab	1b	Metastatic pancreatic cancer	13	Gemcitabine	4 PR (2 unconfirmed) 38% SD Med PFS 5.3 mo	
Kindler 2010 [50]	Conatumumab	2 r.	Metastatic pancreatic cancer	125	Arm 1: conatumumab + gemcitabine Arm 2: AMG 479 + gemcitabine Arm 3: placebo + gemcitabine		Arm 1: 24/38 SD Med PFS: 3.9 mo Arm 2: 1/38 PR, 19/38 SD Med PFS: 5.1 mo Arm 3: 2/40 PR, 13/40 SD Med PFS: 2.1 mo
Paz-Ares 2009 [45]	Conatumumab	1b	Advanced NSCLC	12	Paclitaxel carboplatin	1 CR, 3 PR, 3 SD Med PFS 5.1 mo	

Table 1: continued.

Author	Drug	Phase	Tumor type	No. of patients	Combined with	Best response	Results phase 2 randomized studies
Peeters 2010 [48]	Conatumumab	1b/2	Metastatic colorectal cancer	53	Panitumumab	WT KRAS/MT KRAS 8 SD/4SD Med. PFS 7.3/4.4 mo	
Saltz 2009 [46]	Conatumumab	1b	Metastatic colorectal cancer	12	Modified FOLFOX6 and bevacizumab	5 PR (2 unconfirmed), 6 SD	
Forero-Torres 2010 [23]	Tigatuzumab	1	Relapsed/refractory carcinomas or lymphomas	17	-	7 SD	
Sharma 2008 [26]	LBY135	1	Advanced solid tumors	56	Arm 1: LBY135 Arm 2: LBY135 + capecitabine	Arm 1: 1 minor response Arm 2: 1 PR	
Griffith 2007 [55]	Ad5-TRAIL	1	Prostate cancer	3	-	NR	

NR = not reported, CR = complete response, PR = partial response, SD = stable disease, med PFS = median progression free survival, OS = overall survival, Mo = months, ORR = objective response rate, r = randomized

has also been conducted in 24 pediatric patients with solid tumors. Doses up to 10 mg/kg every 14 days were found to be safe in this population. Five patients achieved stable disease. In one patient stable disease was ongoing at 17 months [21].

Drozitumab was investigated at doses up to 20 mg/kg every 14 days and the maximum tolerated dose was not reached. Twenty out of 41 evaluable patients achieved stable disease. Minor responses were found in 3 patients with colorectal cancer, ovarian cancer and chondrosarcoma with respectively 28%, 23% and 20% reduction in measurable disease [22].

In a phase 1 study evaluating tigatuzumab in doses up to 8 mg/kg iv every week, 7 out of 17 patients experienced stable disease as a best result, with 1 patient having stable disease for over 2 years. The maximum tolerated dose was not reached [23].

Conatumumab was studied in doses up to 20 mg/kg every 2 weeks in 37 patients. The maximum tolerated dose was not reached. One partial response was seen in a patient with NSCLC, who was still on treatment after 4.2 years. One minor response (24% decrease in tumor size) was observed in a patient with colorectal carcinoma and 14 patients achieved stable disease [24]. In another phase 1 study, conatumumab was also well tolerated and 9 out of 18 patients achieved stable disease [25].

LBY135 monotherapy was investigated in doses up to 20 mg/kg every 3 weeks in 32 patients with advanced solid tumors. A minor response in one patient and a decrease in tumor markers in two patients were seen. There were no dose limiting toxicities in patients receiving LBY135 monotherapy [26].

Pharmacokinetic analyses of these antibodies against TRAIL-R2 showed that the serum half life of the antibodies is around 14 days for lexatumumab, 9–19 days for drozitumab and 13–19 days for conatumumab. The plasma half life of tigatuzumab is 6–10 days and preliminary results show a half life value of 10 days for LBY135 [19,20,22–24,26]. In one patient, antibodies against lexatumumab were found before treatment, but this finding was not confirmed in later samples [19]. Antibodies against drozitumab were found in one patient, but because the baseline test of this patient was also positive, this did not seem to be related to treatment [22]. In the study with LBY135, immunogenicity was found in 25% of the patients, which seemed to affect exposure in five patients during later doses [26].

Efficacy of PARAs as single agents

Side-effects in single agent studies were generally mild, with the side-effects seen most frequently being fatigue and nausea. All investigated PARAs were considered safe and well tolerated and a maximum tolerated dose was only found for lexatumumab. From preclinical data there were concerns about a possible toxic effect of PARAs, especially on the liver [27]. However, in the clinical studies so far, this was not confirmed.

Most of the single agent studies had a phase 1 character and were performed in heavily pretreated patients and are therefore not ideal to judge anti-tumor activity. Tumor responses were observed in lymphomas treated with mapatumumab and three partial responses in solid tumors have been reported after treatment with dulanermin and conatumumab. The complete

responses with mapatumumab were achieved at 9 and 11 months, the partial responses with dulanermin at 2 and 8 months and the partial response with conatumumab at 8 months. Interestingly, further decrease in tumor size was seen in some of these patients after about 9 and 11 months (mapatumumab) and 22 months (conatumumab) [13,18,24]. Although these are only individual cases, this indicates that response evaluation with RECIST criteria after the first months of treatment might be underestimating the therapeutic efficacy of PARAs. This underscores the relevance of waterfall plots over time.

The clear difference between the half life values of dulanermin and the agonistic antibodies indicates that dulanermin is only shortly available to bind to death receptors on tumor cells after administration while the antibodies are present for a long time. However, this short availability does not preclude tumor responses. The precise consequences of these differences in half life for trial design are still unknown.

Combination studies

Based on preclinical data there is a strong rationale to combine PARAs with chemotherapy, radiotherapy and other targeted therapies as PARAs enhance their effect [10-12]. These combinations theoretically induce cell death by targeting both the extrinsic and the intrinsic apoptotic pathway. Activation of both the extrinsic and intrinsic apoptotic pathway is amplified by the combination of PARAs with chemotherapy. PARAs amplify signaling of the intrinsic apoptotic pathway via tBid, while chemotherapy augments activation of the extrinsic apoptotic pathway via, among others, TRAIL receptor upregulation at the cell surface and reduction of cellular cFLIP levels. Proteasome inhibition with bortezomib results in pleiotropic effects, but bortezomib treatment is found to induce TRAIL receptor surface expression, reduce FLIP expression, block IAP functionality and prevent proteasomal degradation of p53 and pro-apoptotic Bcl2 family members in cancer cells [28]. Inhibition of the NF-kappaB, Akt or MAPK prosurvival pathways using cetuximab, rituximab or sorafenib synergizes with PARAs targeting the apoptotic pathway. The mechanism of drug interaction can be at the DISC resulting in enhanced DISC formation or more downstream causing reduced expression of anti-apoptotic Bcl-2 family members and IAPs [29,30].

Dulanermin

In a phase 1b study, dulanermin in doses up to 8 mg/kg iv for 5 days or up to 20 mg/kg iv for 2 days every 3 weeks was studied in combination with paclitaxel, carboplatin and bevacizumab. Of the 24 patients with NSCLC included, 1 patient achieved a complete response, 13 a partial response and the median progression free survival was 7.2 months. A maximum tolerated dose was not reached. Combination of dulanermin with these drugs did not significantly affect pharmacokinetics of dulanermin [31]. Results of a randomized phase 2 study with paclitaxel and carboplatin ± bevacizumab ± dulanermin (8 mg/kg iv for 5 days or 20 mg/kg iv for 2 days every 3 weeks) in 213 chemo naïve NSCLC patients showed that this combination is well tolerated. However, this combination did not result in a better objective response rate or progression free survival [32].

Dulanermin in doses up to 8 mg/kg iv for 5 days every 3 weeks was also combined with rituximab during 4 cycles in patients with low-grade NHL. There were 3 complete and 3 partial responses out of 12 patients treated [33]. The preliminary results of a randomized phase 2 study in patients with relapsed follicular NHL did not show a better objective response rate for this combination (61.5% versus 63.6%) [34].

Preliminary results in colorectal cancer patients show that dulanermin (up to 8 mg/kg iv for 5 days every 3 weeks) combined with irinotecan and cetuximab or dulanermin (up to 9 mg/kg iv for 3 days every 2 weeks) with leucovorin, 5-fluorouracil and irinotecan (FOLFIRI) is safe [35].

Mapatumumab

Mapatumumab in doses up to 30 mg/kg iv every 3 weeks was studied in combination with gemcitabine and cisplatin and in doses up to 20 mg/kg iv with paclitaxel and carboplatin in phase 1 studies. The maximum tolerated dose was not reached in either study. Partial responses were observed in respectively 12 out of 49 and 5 out of 27 patients. Combination of mapatumumab with chemotherapy regimens did not seem to influence the pharmacokinetics of any agent [36,37].

Preliminary results from a randomized phase 2 trial of mapatumumab combined with carboplatin and paclitaxel in 111 patients with NSCLC show that this combination does not lead to a better response rate or longer progression free survival [38].

In a phase 1b study, mapatumumab (up to 30 mg/kg every 3 weeks) was combined with sorafenib (400 mg BID) in patients with advanced hepatocellular carcinoma and chronic viral hepatitis. Among 19 patients, a partial response was seen in 2 patients and 4 patients achieved stable disease [39].

Agonistic TRAIL-R2 antibodies

In a phase 1b study, the combination of lexatumumab up to 10 mg/kg every 2 weeks with gemcitabine or FOLFIRI or lexatumumab every 3 weeks with pemetrexed or doxorubicin was studied in 41 patients. Preliminary results show 2 partial responses in colorectal cancer patients in the FOLFIRI arm and 1 partial response in a patient with small cell lung cancer in the doxorubicin arm. Pharmacokinetics of lexatumumab or the chemotherapeutics were not influenced by each other [40].

A randomized phase 2 study in 124 patients was performed comparing drozitumab or placebo plus paclitaxel, carboplatin and bevacizumab in previously untreated patients with NSCLC. The objective response rate did not differ between the two arms (respectively 40% and 42%), nor did the progression free survival [41].

A phase 2 study of drozitumab (10 mg/kg every 3 weeks, after a loading dose of 15 mg/kg) with rituximab in patients with NHL previously treated with rituximab, showed that this combination was well tolerated and 20 out of 40 patients achieved an objective response, consisting of 2 complete responses and 18 partial responses [42].

Two phase 1b studies in metastatic colorectal cancer patients were performed, in which drozitumab was combined with either FOLFOX and bevacizumab or cetuximab and irinotecan

or FOLFIRI ± bevacizumab. All combinations were found to be well tolerated [43, 44].

Conatumumab was studied in combination with several chemotherapy regimens and targeted therapies. In previously untreated patients with advanced NSCLC, the combination of conatumumab (up to 15 mg/kg every 3 weeks) with paclitaxel and carboplatin resulted in 1 complete response and 3 partial responses among 10 evaluable patients. Pharmacokinetics of conatumumab seemed not to be affected by combined treatment with paclitaxel and carboplatin [45].

Combination of conatumumab (up to 10 mg/kg every 2 weeks) with modified FOLFOX6 and bevacizumab in 12 patients with previously untreated colorectal cancer and combination with gemcitabine in 13 previously untreated patients with metastatic pancreatic cancer resulted in partial responses in respectively 5 and 4 patients. Pharmacokinetics showed no differences with those found in single agent studies [46,47].

Conatumumab (10 mg/kg every 2 weeks) in combination with panitumumab in pretreated metastatic colorectal cancer patients appeared to be safe, but did not result in objective responses in either patients with wild-type KRAS tumor status or mutant KRAS tumor status. Stable disease was seen in 8 out of 19 patients with wild-type KRAS and 4 out of 25 patients with KRAS mutant status [48].

Conatumumab (up to 15 mg/kg iv every 3 weeks) was also combined with AMG 479 (an insulin-like growth factor receptor 1 antagonistic antibody) in a phase 1 study. Three out of 9 patients achieved stable disease. Dose limiting toxicities were not observed and no interactions were seen between these agents [49].

In a randomized phase 2 study the combination of gemcitabine with conatumumab (10 mg/kg every 2 weeks) or AMG 479 or placebo in 125 patients with previously untreated pancreatic cancer was studied and showed that these combinations are well tolerated. Although no objective response was seen in the conatumumab arm, stable disease rate, progression free survival and 6 month survival seem to be better in the conatumumab and AMG 479 arms compared to placebo [50].

In a phase 1/2 open-label and double blind study in patients with metastatic or unresectable soft tissue sarcomas, patients were given conatumumab (15 mg/kg every 3 weeks) with doxorubicin or placebo with doxorubicin. Although this combination was safe, the addition of conatumumab did not improve the progression free survival or the response rates [51].

In the phase 1 trial with LBY135, 24 patients received LBY135 in doses up to 20 mg/kg every 3 weeks in combination with capecitabine (2 times daily, 2 weeks on, 1 week off). In these patients, 1 partial response was seen [26].

Efficacy of PARAs in combination studies

Data of 6 randomized phase 2 studies are available. The results indicate that only the combination of conatumumab and gemcitabine shows a trend toward a longer progression free survival and 6 month overall survival [50]. Addition of dulanermin to rituximab in NHL patients

does not seem to improve the objective response rate compared to rituximab alone, nor did the addition of conatumumab to doxorubicin in soft tissue sarcoma patients [34,51].

There were 3 randomized studies in patients with NSCLC. Combination of paclitaxel and carboplatin with mapatumumab does not seem to improve the objective response rate or progression free survival compared to chemotherapy alone. The same was the case for dulanermin and drozitumab with paclitaxel, carboplatin and bevacizumab [32,38, 41]. Therefore regrettably no benefit of the addition of dulanermin, mapatumumab or drozitumab could be shown in these randomized phase 2 studies.

However, we know that the combination of cetuximab or panitumumab with bevacizumab and chemotherapy in colorectal patients performed worse than one of the antibodies separately [52,53]. Therefore the addition of other antibodies to bevacizumab may hide its anti-tumor activity. The reason for this is still unraveled; however bevacizumab effects on tumor vascularization could be involved in this.

Interpretation of the preliminary results of the non-randomized phase 2 combination studies is hampered by the limited size of the studies. In earlier phase 1 combination studies, waterfall plots show anti-tumor effects of treatment with PARAs, although responses often do not meet the formal current RECIST criteria for partial response [31,36,37,54].

FUTURE PERSPECTIVES

Ongoing studies

In Table 2, the diverse ongoing studies with PARAs are shown. Of special interest are the studies that combine PARAs with other targeted therapies, since targeting the apoptosis route on multiple levels might lead to improved effectiveness.

Although PARAs also enhanced the cytotoxic effects of irradiation in preclinical settings, no clinical trials investigating the combination of these agents with radiotherapy have been conducted to date. A phase 1b/2 study combining mapatumumab, radiotherapy and cisplatin in patients with advanced cervical cancer (NCT01088347) and a phase 1/2 study combining conatumumab with gemcitabine, capecitabine and radiation therapy in patients with pancreatic cancer (NCT01017822) have been initiated.

Another approach is direct injection of recombinant adenovirus that encodes for TRAIL in tumor tissue. In a phase 1 trial these injections were given in the prostate of patients with prostate cancer. Preliminary results of the first 3 patients show that the injection was well tolerated [55].

Novel PARAs

In recent years several novel PARAs with improved properties targeting TRAIL receptors have been developed. Fusion of an antibody derivative to TRAIL can result in antibody targeting-dependent activation of TRAIL and other TNF family members that are in their soluble form biologically less active [56]. A number of TRAIL fusion proteins have been constructed, where recombinant soluble TRAIL was genetically linked to a receptor selective antibody fragment

Table 2: Ongoing trials with pro-apoptotic receptor agonists.

Drug	Phase	Tumor type	Combined with/study arms
Dulanermin	1b	Metastatic colorectal cancer	FOLFOX and bevacizumab
Mapatumumab	1b/2	Advanced cervical cancer	Cisplatin and radiotherapy
Mapatumumab	2	Advanced hepatocellular carcinoma	Arm 1: sorafenib + placebo Arm 2: sorafenib + mapatumumab
Mapatumumab	2	Relapsed/refractory multiple myeloma	Arm 1: bortezomib Arm 2: bortezomib + mapatumumab 10 mg/kg Arm 3: bortezomib + mapatumumab 20 mg/kg
Lexatumumab	1	Solid tumors/lymphoma	Recombinant interferon gamma
Conatumumab	1b	Lymphoma	Arms 1, 3, 5, 7: bortezomib + conatumumab Arms 2, 4, 6: vorinostat + conatumumab
Conatumumab	2	KRAS mutant metastatic colorectal cancer	Arm 1: conatumumab + FOLFIRI + AMG 479 placebo Arm 2: AMG 479 + FOLFIRI + conatumumab placebo Arm 3: FOLFIRI + AMG 479 placebo + conatumumab placebo
Conatumumab	1/2	Pancreatic cancer	Gemcitabine hydrochloride, capecitabine and radiation therapy
Conatumumab	1b/2	Metastatic colorectal cancer	Arm 1: conatumumab (low dose) + mFOLFOX + bevacizumab Arm 2: placebo + mFOLFOX + bevacizumab Arm 3: conatumumab (high dose) + mFOLFOX + bevacizumab
Conatumumab	2	Solid tumors/lymphoma	FOLFOX6, ganitumumab, bevacizumab (open label extension study)
Tigatuzumab	2	Pancreatic cancer	Gemcitabine

Drug	Phase	Tumor type	Combined with/study arms
Tigatuzumab	2	NSCLC	Arm 1: carboplatin + paclitaxel + tigatuzumab Arm 2: carboplatin + paclitaxel + placebo
Tigatuzumab	2	Metastatic colorectal cancer	Arm 1: irinotecan + tigatuzumab Arm 2: irinotecan
Tigatuzumab	2	Advanced liver cancer	Arm 1: sorafenib + tigatuzumab Arm 2: sorafenib
Tigatuzumab	2	Ovarian cancer	paclitaxel + carboplatin
Tigatuzumab	1	Metastatic colorectal cancer	FOLFIRI
Tigatuzumab	2	Metastatic triple negative breast cancer	Arm 1: paclitaxel protein-bound + tigatuzumab Arm 2: paclitaxel protein-bound

Source: clinicaltrials.gov, August 2011.

based on a single chain variable fragment (scFv). The scFv can be directed against cancer specific targets such as EGFR [57], against AML cells using anti-CD33 scFv and against acute leukemic T-cells using anti-CD7 scFv [58,59].

Another approach to raise more effective PARAs is the computational design of TRAIL variants that bind TRAIL-R1 or TRAIL-R2 with stronger affinity and preferably have reduced affinity to the decoy receptors compared with rhTRAIL [60,61]. A novel antibody based approach is a tetrameric nanobody agonist targeting TRAIL-R2, TAS266 (Novartis) [62].

Biomarkers

Another important subject of intense investigation is the search for biomarkers that can predict the tumor response to these new agents and could thus be used to personalize treatment.

It would be of major interest to know whether apoptosis induction via TRAIL-R1 or TRAIL-R2 is influenced by the expression of these receptors and cellular downstream proteins in the tumors. Just immunohistochemistry of these targets in the tumor tissue may not be enough, since no clear relation between receptor expression and outcome has been shown in clinical studies so far [16-19, 36,37]. Other potential biomarkers for apoptosis are also evaluated in clinical trials [63,64]

In the preclinical setting the rate of O-glycosylation of TRAIL-R1 and TRAIL-R2 appears to be predictive of the sensitivity of tumor cells to dulanermin and drozitumab. In dulanermin and drozitumab sensitive tumor cells higher expression of mRNA encoding enzymes involved in O-glycosylation was found [65,66]. These enzymes can be assessed using immunohistochemistry assays, which are now tested in clinical trials with PARAs [32, 67].

Antibodies that are (radio)labeled could possibly also be used as biomarkers [68-72]. Preliminary results of an imaging study with ¹¹¹Indium labeled mapatumumab in patients show that mapatumumab is taken up in part of the tumor lesions [73]. An imaging trial with ¹¹¹Indium labeled CS1008 (tigatuzumab) is currently ongoing (NCT01220999). These imaging techniques could potentially predict availability of the drug at the tumor site and guide future therapy.

CONCLUSION

Based on its property to induce apoptosis in tumor cells while sparing normal cells, PARAs are of interest to explore as a new cancer treatment modality. In clinical studies, the use of both rhTRAIL and antibodies against TRAIL-R1 and TRAIL-R2 appears to be safe and side effects are generally mild. Monotherapy with these agents resulted in some anti-tumor efficacy which could occur after a long treatment period.

Combination of PARAs with other treatments seems to be safe. Although no full phase 3 studies have been performed, all results until now show only modest effects. The maximum tolerated dose with these (combinations of) drugs was mostly not reached. This uncertainty about dosing could partly be addressed by molecular imaging and labeling of the drugs involved. If there is a role for these drugs, it will be in the setting of a rational combination therapy. Ongoing

(randomized) combination studies, including combinations with other targeted therapies and radiotherapy, are awaited. Furthermore, novel PARAs with improved properties targeting TRAIL receptors and new biomarkers and imaging strategies that may help to select patients might lead to higher response rates in future trials.

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