A fusion protein between a 5T4 binding antibody fragment and an engineered superantigen (ANYARA) is a targeted immunotherapy effective alone and in combination with other cancer therapies

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Introduction

In this study we explored the possibility of combining antibody targeted immunotherapy against cancer with other established tumor therapies such as chemotherapy or anti-angiogenic therapy, i.e. docetaxel, bevacizumab and sunitinib. Antibody targeting of superantigens to tumor cells combines powerful T cell activating property, a potent cytotoxic activity with a targeted approach to eradicate tumor cells (Figures 1 and 2). Tumor targeted superantigens (TTSs) are recombinant fusion proteins that consist of an anti-tumor Fab moiety genetically fused to a superantigen. TTSs are given intravenously in cycles of 4-5 daily injections. However, clinical practice has identified several issues that need to be addressed to optimize such molecules. Naptumomab estafenatox (ANYARA) has been designed based on the experience from predecessor products in clinical trials. Critical properties such as tumor reactivity and therapeutic window were improved. An engineered Fab moiety recognizes the 5T4 antigen expressed on a large number of solid tumor forms with an affinity in the order of 1 nM. The fusion protein induces T cell mediated killing of tumor cells at concentrations around 10 pM and the superantigen moiety has been engineered to have low binding to human antibodies and MHC class II.

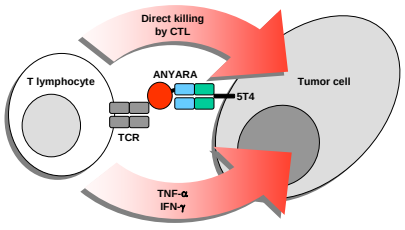

Figure 1. Mechanism of action for ANYARA.

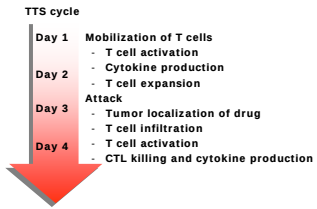

Figure 2. Mechanism of action for ANYARA. ANYARA is given in cycles of 4-5 daily i.v. injections.

The 5T4 antigen is expressed on many types of carcinomas including more than 95% of tumors from patients with non-small cell lung (NSCLC), renal cell (RCC) and pancreatic cancer (PC) (Table 1). In clinical phase I dose escalation studies the MTD was estimated to be 15 and 22 microg/kg in patients with RCC and NSCLC/PC, respectively. ANYARA had expected and manageable side effect profile exhibiting transient fever, chills, nausea, vomiting and hypotension as common AEs. Dose-dependent production of cytokines such as IL-2 and IFN-gamma was recorded as well as selective expansion of Vbeta6.4 expressing T lymphocytes. In clinical PET studies ^{124}I-labeled ANYARA has been shown to localize to 5T4 positive tumors. Efficacy shown as e.g. overall survival was also encouraging in the phase I trials (Figure 3). ANYARA is in phase III clinical development of RCC.

	Percent 5T4$^+$ pts
NSCLC	>95
Renal cell cancer	>95
Pancreatic cancer	>95
Prostate cancer	>95
Breast cancer	85-95
Cervix cancer	85-90
Ovarian cancer	>70
Gastric cancer	50
Colorectal cancer	40-50

Table 1. Expression of the 5T4 tumor antigen in different carcinomas.

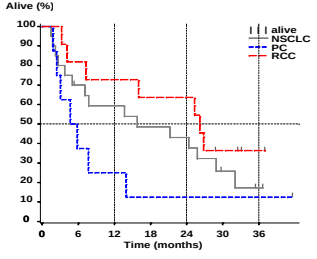

Figure 3. Overall survival for the advanced PC (n=8), RCC (n=11) and NSCLC (n=20) patients in the phase I dose escalation studies with ANYARA. Median overall survival times were 5.2 months for PC, 15.8 months for NSCLC and 26.2 months for RCC.

Materials and Methods

- Tumor model: SCID mice were inoculated i.p. with the human tumors Caki-2 (human renal cell carcinoma), Calu-1 (human non-small cell lung carcinoma) or Colo205 (human colon carcinoma). These human tumors naturally express the 5T4 tumor antigen.
- ANYARA-treatment: Mice were routinely given superantigen-activated human T lymphocytes i.p. in association with the first ANYARA injection in cycles of 4-5 daily i.v. injections with 5 or 50 microg/mouse/day of ANYARA.
- Docetaxel was administered i.p. with 200 microg/mouse at day 7 after tumor inoculation.
- Bevacizumab was administered i.p. with 100 microg/mouse/day at days 1, 4, 8, 11, 15, 18, 22 and 25 after tumor inoculation.
- Sunitinib was administered p.o. with 200 microg/mouse/day at days 1-25 and 30-54 after tumor inoculation.
- The experiments were terminated at days 27 (Colo205), 50 (Calu-1) or 55 (Caki-2) and the animals were inspected for tumor growth, tumors were recovered and weighed.

Results

SCID mice inoculated with the human renal cell carcinoma Caki-2 were treated with 1 or 2 cycles of activated human T lymphocytes (i.p.) day 5 or days 5 and 26 and 50 microg of ANYARA (i.v.) days 5-8 or 5-8 and 26-29. A marked reduction in tumor mass was achieved after 1 cycle of ANYARA treatment and 2/8 mice were tumor free. Only minute tumor mass was recovered and 4/8 mice were tumor free after 2 cycles of ANYARA (Figure 4).

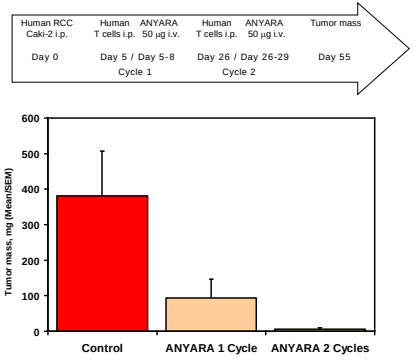

Figure 4. Human Caki-2 tumor mass in SCID mice after treatment with 1 or 2 cycles of ANYARA.

SCID mice inoculated with the human non-small cell lung carcinoma Calu-1 were treated with 200 microg of docetaxel (i.p.) at day 7, 1 cycle of activated human T lymphocytes (i.p.) day 2 and 50 microg of ANYARA (i.v.) days 2-6, or the combination. A marked reduction in tumor mass was achieved after treatment with docetaxel or ANYARA. The combination resulted in additive anti-tumor effects (Figure 5).

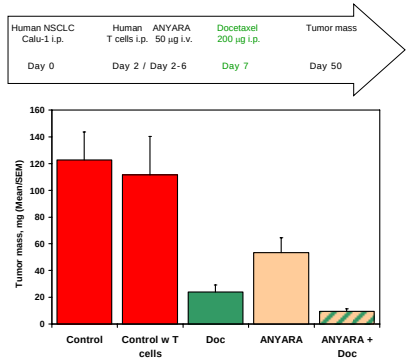

Figure 5. Human Calu-1 tumor mass in SCID mice after treatment with docetaxel (Doc) or 1 cycle of ANYARA or the combination.

SCID mice inoculated with the human colon carcinoma Colo205 were treated with a series of 100 microg bevacizumab (i.p.) injections, 1 cycle of activated human T lymphocytes (i.p.) day 5 and 50 microg of ANYARA (i.v.) days 5-8, or the combination. A marked reduction in tumor mass was achieved after treatment with bevacizumab or ANYARA. The combination resulted in additive anti-tumor effects (Figure 6).

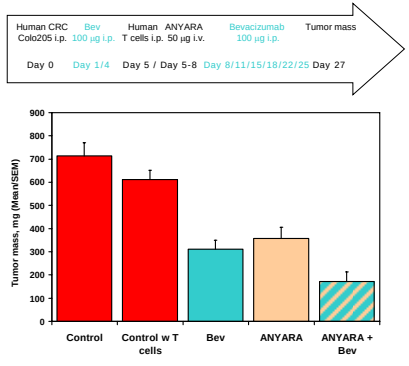

Figure 6. Human Colo205 tumor mass in SCID mice after treatment with bevacizumab (Bev) or 1 cycle of ANYARA or the combination.

SCID mice inoculated with the human renal cell carcinoma Caki-2 were treated with 200 microg of sunitinib (p.o.) days 1-25 and days 30-54, 1 cycle of activated human T lymphocytes (i.p.) day 26 and 5 microg of ANYARA (i.v.) days 26-29, or the combination. A marked reduction in tumor mass was achieved after treatment with sunitinib or ANYARA. The combination resulted in additive anti-tumor effects (Figure 7).

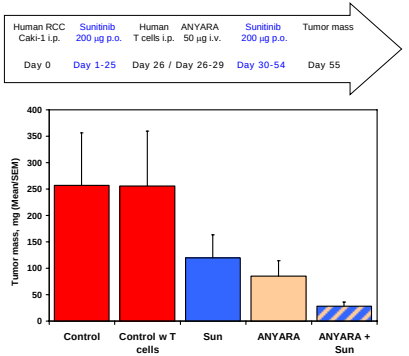

Figure 7. Human Caki-2 tumor mass in SCID mice after treatment with sunitinib (Sun) or 1 cycle of ANYARA or the combination.

Conclusion

ANYARA has the capacity to eradicate established human tumors in SCID mice grafted with human lymphocytes. Here we also show that it can be effectively combined with other established tumor therapies such as chemotherapy or anti-angiogenic therapy, i.e. docetaxel, bevacizumab and sunitinib. ANYARA is in phase III clinical development of RCC and these results give new opportunities on its use in future cancer treatment.

Additional Information

In addition to phase I dose escalation studies as monotherapy ANYARA has been tested in clinical trials in combination with docetaxel (Cohen RB, Borghaei H, Langer CJ, Alpaugh K, Lassen U, Afanasiev BV, Orlov SV, Yablonsky PK, Forsberg G, Hedlund G. An open label phase I study of ABR-217620 in combination with docetaxel in patients with NSCLC. In: AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics; 2007 Oct 22-26; San Francisco, CA. Philadelphia (PA): AACR; 2007. Abstract nr A163). The results show full compatibility of the combination and encouraging efficacy.