Therapeutic targeting of NG2 proteoglycan with mAb and pre-armed NK cells in human GBM evaluated with dynamic enhanced and diffusion weighted MRI in rats

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Introduction: Glioblastoma multiform (GBM) is a highly aggressive brain tumor where the patients have median survival of only 14.6 months with the best available treatment. We have previously shown that GBMs that express high levels of the NG2 receptor (a marker for stem cells) are more angiogenic, treatment resistant and ultimately these patients have a shorter overall survival[1-3]. We aimed to target NG2 with monoclonal antibodies (mAb) in combination with *in vitro* activated natural killer cells (NK) in an adoptive cellular immunotherapy approach. NK cells are large granular lymphocytes involved in both innate and adaptive immune responses and are highly capable of killing tumor cells. In this study, we characterize early treatment responses by multiparametric MRI including dynamic-enhanced MRI (DCE-MRI) and diffusion weighted MRI, and identify quantifiable MRI parameters that may reveal clinically relevant, tumor physiological changes that may be apparent before structural changes are evident. To facilitate personalized medicine, it is important to highlight imaging parameters that may be useful for detecting early signs of treatment failure during patient follow up.

Materials and Methods: Athymic rats were implanted with U87 NG2 expressing GBM cells and divided into four treatment groups: mAb (n=9); NK (n=12); combined NK+mAb (n=8) and controls that received no treatment (n=8). Treatment was given 14 days after implantation. MRI was performed two time points, 7±2 and 17±1 days after treatment start on a 7T Bruker Pharmascan (Bruker Biospin, Germany) with a 38 mm volume coil for transmit and receive. Rats were anesthetized with 1-2% isoflurane in 1:2 O_2/N_2. The MR-protocol included T2-weighted RARE (TR=2400ms, TE=40ms, pixel size=137x137µm^2), ADC-map (TR=1500ms, TE=35ms, pixel size=273x273µm^2, four b-values (300-1200s/mm^2, 3 directions), RARE T1-map (TR=125-10 000ms, TE=12.5ms, pixel size=273x273µm^2), RARE T1-weighted pre contrast (TR=1300, TE=8.86, pixel size=137x137µm^2), RARE T1-weighted dynamic scan (TR=200ms, TE=8.86ms, 206 repetitions, pixel size=273µm^2, 0.2mmol/kg Omniscan injected through the tail vein after 20 baseline scans) and RARE T1-weighted post contrast image as above. Four slices was used in T1-maps and dynamic scan, while eight slices were used in all other scans. A slice thickness of 1.5mm was used in all scans. The total scan time was approximately 80 min. Follow-up MRI was performed on surviving animals 3 months after treatment start (T1-weighted pre- and post-contrast images only). Tumor sections were immunohistochemically stained for various markers involved in angiogenesis, growth and death of the tumor. MR data analysis was done using in-house build programs written in Matlab7.8.0 (The Mathworks, Inc). By assuming a two-compartment model, the volume transfer constant between blood plasma and extra-vascular extracellular space (EES), K^{trans}, volume of EES, v_e, volume of blood plasma, v_p, relative signal intensity 2 minutes after contrast injection, RSI_{2min}, area under the curve during the two first minutes after contrast injection, AUC_{2min} and time to peak, *TTP* were calculated based on the DCE-MRI data. Groups were compared using parametric or non-parametric t-tests and found statistically different if $p<0.05$.

Results: T1-weighted pre contrast images clearly displayed the tumor (Fig 1a). Mean tumor size increased from 7 to 17 days after treatment start in all groups with no significant difference between groups due to large variations within the groups. Follow-up MRI was performed on surviving animals (NK+mAb group only) 3 months after treatment illustrated reduction in tumor volume at that time. Overall survival fraction was significantly increased in the combined NK+mAb treated group only (p=0.02, fig 1d). The distribution volume of EES, v_e, was significantly reduced in rats treated with NK compared to controls at both time-points after treatment start (p=0.031 and 0.040, respectively, figure 1e). Furthermore, AUC was significantly reduced in rats treated with NK compared to controls 1 week after treatment start (p=0.024, figure 1f). DCE-analysis showed no other differences between groups. Also, no differences between groups were found on ADC-maps. Immunohistochemical analysis showed significantly increased apoptosis in rats treated with NK+mAb compared all other groups ($p<0.05$, fig 1b). Furthermore, the cell proliferation was reduced in the same group compared to all other groups ($p<0.001$, fig 1c).

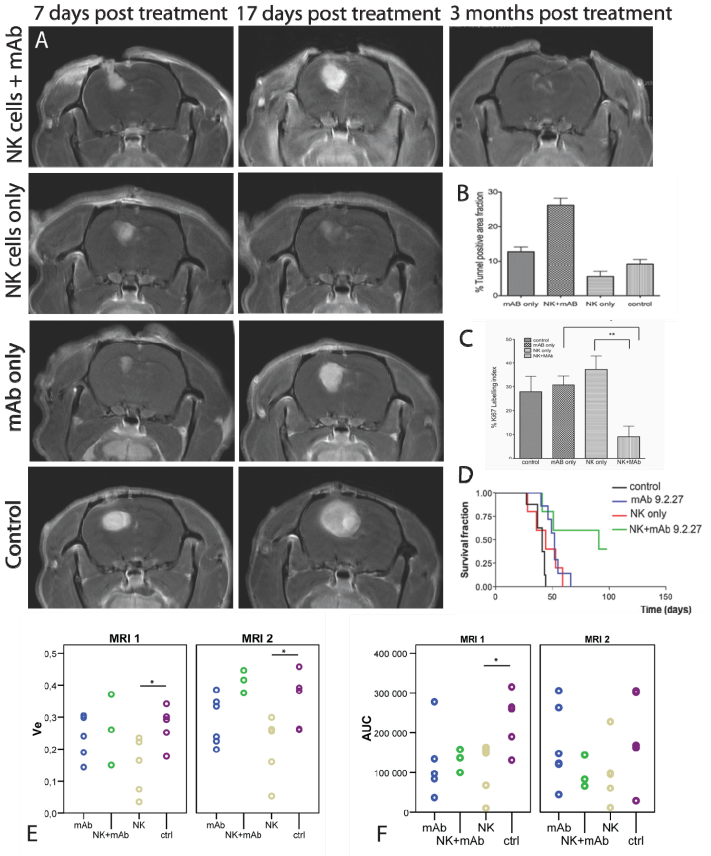

Figure 1: T1-weighted post injection MRI illustrated tumor growth in all groups (a). Apoptosis (b) and cell proliferation (c) was measured by immunohistochemisty and demonstrated significantly differences in NK+mAb treated rats compared to all other groups. Survival was significantly increased in NK+mAb compared to control (d). DCE-MRI demonstrated significant reduction in v_e in rats treated with NK at both time point (e), and in AUC at the first time point only (f).

Discussion and Conclusion:

Overall survival, as well as histological analysis illustrate that the combined treatment with NK and mAb had the greatest therapeutic effect. When NK cells was present in the tumour, the volume of EES was reduced, as illustrated by DCE-analysis. In case of NK+mAb combined treatment, NK cells are still present in the tumour, but no difference from controls are found. This can indicate a reduction in the number of tumor cells present in the solid tumor mass due to the effect of the treatment, which is confirmed by histology and overall survival improvement in this group. This illustrate that mAb must be present and bind to tumor cells to facilitate the effect of NK. Moreover, the combination of monoclonal antibodies and natural killer cells is the most effective treatment for GBMs. Furthermore, multiparametric imaging and DCE-MRI can detect early treatment response and facilitate therapeutic intervention.

References: [1]Chekenya M, Hjelstuen M, Enger PØ, Thorsen F, Jacob AL, Probst B, Haraldseth O, Pilkington G, Butt A, Levine JM, Bjerkvig R, FASEB J. 16:586-8, 2002. [2]Brekke C, Lundervold A, Enger PØ, Brekken C, Stålsett E, Pedersen TB, Haraldseth O, Krüger PG, Bjerkvig R, Chekenya M, Neuroimage 29:965-76, 2006. [3]Chekenya M, Krakstad C, Svendsen A, Netland IA, Staalesen V, Tysnes BB, Selheim F, Wang J, Sakariassen PØ, Sandal T, Lønning PE, Flatmark T, Enger PØ, Bjerkvig R, Sioud M, Stallcup WB, Oncogene 27:5182-94, 2008.