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Investigations on the combined effects of EGFR signaling inhibition, integrin inhibition, and radiation therapy in the treatment of glioblastoma multiforme in vitro and in vivo.

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Introduction and Methods: Glioblastoma multiforme is a mostly incurable, terminal disease with limited treatment options despite tremendous research efforts; new drugs (biologicals) inhibiting more specific signals and drug combinations may offer increased therapeutic efficacy in the coming years. Promising strategies in GBM treatments include EGFR and integrin signaling inhibition, specifically in combination with radiation. Therefore, the aim of this work was to analyze the logical next step, namely to investigate the effects of combinations of EGFR and integrin inhibition with simultaneous radiation in a triple therapy schedule.
To this end, in vitro proliferation assays, clonogenic assays, tube formation assays and Matrigel migration/invasion assays with a combination regimen of cetuximab (a monoclonal antibody to EGFR), cilengitide (an αvβ3 and αvβ5 integrin inhibitor) and radiotherapy were conducted with U87 MG as well as HDMVEC endothelial cells.
In vivo, human glioblastoma cells (U87 MG) were injected into the hind limbs of athymic mice. Established tumors were treated with all combinations of the three treatment modalities. Primary endpoint was the tumor growth delay (TGD). Secondary endpoints were noninvasive radiological imaging methods including magnetic resonance imaging (MRI), flat panel Volume-CT (fpV-CT) and ultrasonic imaging for morphological and functional assessments, e.g. using contrast enhanced ultrasound blood perfusion measurements.
Furthermore, tumors were analyzed for histology and IHC including H&E, CD31/α-SMA and DAPI/Ki-67 stains
Results: In both U87 and endothelial cells, all monotherapies induced proliferation/cell viability inhibition and reduced clonogenic survival. In ECs, all monotherapies reduced migration and tube formation. In general, all dual therapy combinations increased these effects, and the combination of all treatments in a triple combination was the most effective regimen. Interestingly, the cetuximab/cilengitide combination effect was supra-additive for proliferation and clonogenic assays.
In vivo, the subcutaneously growing U87 tumors showed a significant reduction in growth achieved by either monotherapy; the dual therapies again increased tumor growth delay (TGD) vs. the monotherapies, and the triple therapy was the most effective treatment. The TGD analysis showed a supra-additive effect for the combination of cilengitide and cetuximab. Similarly, Kaplan-Meier analysis showed a trend towards improved survival achieved by combination therapies reaching statistical significance by triple therapy vs. controls, although the experiments in the s.c. model had initially not been designed for survival analysis.
The Ki-67 Tumor proliferation index correlated well with tumor growth delay revealing e.g. supra-additive antiproliferative effects by the cilengitide/cetuximab combination. Similarly, the H&E stain exhibited reduced cellularity and increased necrosis/apoptosis in qualitative good correlation to the tumor growth curves. The CD31/SMA stain revealed reduced MVD achieved by Cetuximab and cilengitide; these effects were further enhanced by the triple

combination, suggesting antiangiogenic effects. At the same time and despite the reduced overall MVD, the data indicated a distinct vessel normalization effect in particular by the combination treatments reflected by increased pericyte coverage of the endothelial cells.
The radiological images including MRI and V-CT correlated well with tumor sizes as measured by calipers, and allowed for qualitative analysis of the tumor structure. It was possible to exclude major tumor necrosis by T2-weighted MRI; V-CT images allowed for a qualitative description of the tumor vessels. The ultrasound perfusion study further revealed a quantitative decline in the intratumoral blood volume after monotherapies which was more pronounced towards the combination therapies; together with tumor sizes and histology this reflected most likely overall antiangiogenic treatment effects associated with vascular normalization.
Conclusion: A supra-additive effect of the combination therapy with cetuximab, cilengitide and radiation therapy was found in vitro and in vivo. The animals tolerated the treatment well. In addition, both drug agents have already been used in humans with tolerable toxicity and side effects. Given the rationale of the combination and the effects seen in this preclinical study, a potential clinical benefit of the suggested combination can be expected. Bearing in mind that the first therapy today for newly diagnosed GBM is surgery with other treatments coming in addition, a clinical phase II trial comparing the combination of cetuximab, cilengitide and radiotherapy to the current standard (the combination of radiotherapy and temozolomide) is currently being initiated under the name GERONIMO.