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Overexpression of Eukaryotic Initiation Factor 5a (eIF-5A) and Hypusine Forming Enzymes in Glioblastoma Patient Samples and Therapeutic Potential of Hypusine Modification for Treatment of Glioblastoma Multiforme

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The eukaryotic initiation factor 5A (eIF-5A) has been associated with various malignant neoplasms, however its role and use as a potential target in glioblastoma multiforme has not been elucidated. eIF-5A contains the unique modification hypusine, which is a post-translational modification of lysine that is formed by the enzymes deoxyhypusine synthase (DHS) and deoxyhypusine hydroxylase (DOHH).
Using immunohistochemistry, expression levels of DHS, DOHH, eIF-5A and eIF-5A2 (an isoform with restricted tissue specificity) were investigated in a cohort of 173 gliomas, arranged on a tissue microarray. Two glioblastoma cell lines (g55T2 and U87-MG) were treated with GC7, an inhibitor of DHS and cell proliferation was determined by trypan blue exclusion. The hypusine status of eIF-5A was analyzed using 2D-Western-blots and incorporation of ^{3}H-labeled spermidin into eIF-5A. Knockdown of eIF-5A was performed by lentiviral transduction with specific shRNAs.
Elevated expression levels of DHS, DOHH and eIF-5A have been found in 173 glioma samples with different grades. eIF5A was demonstrated to be overexpressed without significant difference in tumours of all grades, whereas eIF-5A2 was only detected in one single tumour and in persisting neurons. Interestingly, DHS and DOHH were significantly upregulated in glioblastoma samples compared to tumours of grade I-III. In *in vitro* studies, the DHS inhibitor GC7 is able to inhibit formation of hypusinated eIF-5A in the two glioblastoma cell lines U87-MG and g55T2 and reduced proliferation of these glioblastoma cell lines in a dose dependent manner, while normal human astrocytes were not affected by GC7 treatment. Knockdown of eIF-5A by shRNA resulted in a slightly less reduced effect on proliferation. In combination with clinically relevant alkylating agents Temozolomid (Temodal®) and BCNU (Carmustine/BiCNU®), treatment with GC7 had an additive effect on proliferation in these cell lines compared to single treatment.
The elevated expression of eIF-5A, DHS and DOHH in glioma and particularly in glioblastoma tissue samples from patients as well as in two glioblastoma cell lines suggest an important role of these proteins in tumour formation and/or progression. This hypothesis could be confirmed *in vitro* by pharmacologically interfering with hypusine formation or knockdown of eIF-5A in cell lines. Thus eIF-5A and especially the hypusine forming enzymes might comprise promising targets for glioblastoma therapy.

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Functional Comparison of IGF-1R Antibodies and Possible Implications for Clinical Safety and Efficacy

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Background: This is the first study performing a head to head comparison of monoclonal IGF-1R antibodies (mAb) based on published sequences for therapeutic mAbs from Pfizer (CP751,871, IgG2 kappa), Amgen (AMG479, IgG1 kappa), Merck (h7C10, IgG1 kappa), Imclone (IMC-A12, IgG1 lambda) and Roche (R1507, IgG1 kappa).
Methods: In this study, sequence information for IGF-1R mAbs was extracted from patents and used to clone and transiently express IgGs (*=re-synthesized) in HEK293F cells. *In vitro* assays for ligand binding, IGF-1R auto-phosphorylation, IGF-1R downregulation, IR co-downregulation, and affinity analyses (Biacore) were used. *In vivo* comparison was done in BxPC3 xenograft mouse model.
Results: All antibodies inhibit IGF-1 binding and signaling at low nanomolar levels. While IMC-A12* and R1507 also prevent IGF-2 binding to IGF-1R and subsequent receptor activation, CP751,871* and h7C10* do not inhibit IGF-2 binding and have only limited impact (55%/30% inhibition) on IGF-2 signaling.
Analysis of IGF-1R phosphorylation in 0.5% FCS medium revealed that all antibodies except R1507 exert agonistic activity. Interestingly, Fab fragments of agonistic mAbs became antagonistic, indicating that bivalent binding is necessary for agonistic effects.
All mAb (200nM, 24h treatment of MCF-7 cells) with the exception of AMG479* efficiently downregulated the IGF-1R by 78−82%. Analysis of the same cell lysates revealed however striking differences in IR co-downregulation, a mechanism discussed as possible cause of clinical hyperglycemia and cardiotoxicity. R1507 had the least side effects on Insulin co-downregulation (9%) compared to h7C10* (15%), CP751,871* (23%) and IMC-A12* (46%).
Differences were also seen in the binding kinetics. Both AMG479* and R1507 showed faster k_{on} and k_{off} rates resulting in shorter retention times at the receptor. Since k_{on}/k_{off} rates are discussed to influence tumour penetration (1), we compared downregulation of IGF-1R by R1507 and CP751,871* in xenograft tumours. In combination with Avastin, R1507 was significantly more effective both in downregulating IGF-1R and in inhibiting tumour growth.
Conclusion: The head to head comparison of IGF-1R mAbs revealed differences in regard to binding properties, tumour penetration, IR co-downregulation, and inhibition of signaling via the ligand IGF-2.

References

[1] Adams, G. P., Schier, R., McCall, A. M., Simmons, H. H., Horak, E. M., Alpaugh, R. K., Marks, J. D., and Weiner, L. M. (2001) *Cancer Res.* **61,** 4750–4755

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Eribulin Mesylate Pharmacokinetics in Patients With Advanced Solid Tumours Receiving Rifampicin

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Background: Eribulin mesylate (Halaven™), a non-taxane microtubule dynamics inhibitor with a novel mechanism of action, has clinical activity in breast cancer and other solid tumours. This study assessed the effects of CYP3A4 induction by rifampicin on eribulin pharmacokinetics (PK).
Methods: This uncontrolled, open-label, non-randomized, crossover Phase I study (EudraCT 2009–013430−24; sponsored by Eisai Ltd; completed) enrolled 14 patients (⩾18 years) with advanced solid tumours that had progressed after standard therapy or for which none existed. In a 28-day cycle, patients received eribulin mesylate 1.4 mg/m^2 2−5 min IV infusion on Days 1 and 15. Oral rifampicin 600 mg was administered once-daily from Days 9 to 20. Plasma samples were collected over 144 h post-eribulin dose on Days 1 and 15. PK parameters were calculated using non-compartmental analysis. Area under the curve ($AUC_{0-\infty}$) and maximum plasma concentration (C_{max}) values were analyzed using analysis of variance, and mean ratios and 90% confidence intervals (CI) were calculated for eribulin + rifampicin vs eribulin alone. No interaction was declared if 90% CI were within 70–143%. Safety was also assessed.
Results: In 11 PK-evaluable patients, $AUC_{0-\infty}$ following eribulin administration alone was similar to that of eribulin + rifampicin (ratio of geometric least-square (GLS) means: 1.10, 90% CI 0.91, 1.34), and C_{max} was also similar (ratio of GLS means: 0.97, 90% CI 0.81, 1.17). Rifampicin had no effect on eribulin clearance (CL) or elimination half-life ($t_{1/2}$). For eribulin vs eribulin + rifampicin, mean CL was 3.41 vs 3.18 L/h, respectively; mean $t_{1/2}$ was 40.36 vs 36.64 h, respectively. In the safety population, the incidence of patients with ⩾ grade 3 treatment-related adverse events (AEs) was 2 (14.3%) for eribulin alone (during Days 1−8; n = 14) and 4 (33.3%) for eribulin + rifampicin (after Day 9 or first dose of rifampicin; n = 12). The combination has a longer exposure time so more AEs were expected, and AEs after Day 9 could also have been due to the Day 1 eribulin dose. There were no deaths or life-threatening serious AEs reported as treatment-related, and there was one treatment-related AE leading to study withdrawal per group.
Conclusions: Coadministration of rifampicin with eribulin had no effect on eribulin disposition, indicating that eribulin can be safely coadministered with CYP3A4 inducers. Eribulin was generally safe and well tolerated.