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The use of patient-derived 3D cell cultures in pre-clinical research

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Together with our partner company EPO we have generated patient-derived 3D (PD3D™) cell culture models and corresponding xenografts from more than 120 patients with primary or metastatic cancers of various origins. Although early diagnosis and molecular characterization of cancer has improved significantly, methods for rapid and cost-effective prediction of an optimal and individualized treatment are still missed. We present an experimental pipeline combining molecular genotyping and experimental drug testing for the individual patient. Starting from Matrigel-based Patient-derived three-dimensional PD3D™ cell cultures, we were able to establish numerous long-term PD3D™ cell cultures suitable as models for basic and translational research. Immunohistochemistry analyses demonstrated, that our in vitro cultures preserve an in vivo-like architecture, preventing tumor cells from differentiating and allowing the investigation of intra-tumor heterogeneity and cancer stem cell-like sub-populations. To interrogate the mutation status of selected clinically relevant oncogenes and tumor suppressors in PD3D™ cultures, we applied cost-efficient benchtop sequencing and show the preservation of putative driver mutations found in the original tumor. IC50 data generated by automated 384-well based dose-response experiments with approved drugs are then used to link individual genotypes with drug sensitivity phenotypes. These drug sensitivity profiles serve as a source of comparison and complementation to drug response data of the corresponding in vivo PDX models or even patients, where applicable. These wet-lab data, combined with in vivo and clinical data, will serve as a basis for both, early phase drug development including companion diagnostics to new predictors for tailored therapies.

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Therapeutic enzymes for the treatment of leukemia: Molecular engineering of L-asparaginases to improve activity and stability

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This lecture will highlight the development of novel strategies for the amelioration of the enzyme L-asparaginase (L-ASNase) which is a protein drug of high value in antileukemic therapy. L-ASNases catalyze the deamidation of the free amino acid L-asparagine (L-Asn) to L-aspartate (L-Asp) and ammonia. Bacterial L-ASNases are FDA-approved therapeutic enzymes for use in the treatment of various blood cancers to deplete serum L-Asn levels. Their efficacy as protein drugs is based on the fact that several hematological malignancies, in particular Acute Lymphoblastic Leukemia (ALL), depend for growth on the extracellular supply of L-Asn. To avoid the immune response and other side reactions inherent to the bacterial enzymes, it would be beneficial to substitute them with human L-ASNases. One human isoform, hASNase-3, belongs to the N-terminal nucleophile (Ntn) hydrolase superfamily where the protein is synthesized as a single polypeptide chain that is devoid of activity. Autoproteolytic cleavage of this protein generates two tightly associated subunits that constitute the catalytically active enzyme. The free amino acid glycine was found to selectivelyaccelerate intramolecular processing of hASNase-3 both in vitro and in human cells. We evolved the enzyme in vitro aiming to select for variants of enhanced activity. Since increased expression of hASNase-3 was observed in several tumors, the dependence of hASNase-3 activation on glycine may be related to the altered metabolic profile of cancer cells.To increase the serum half-life of the enzyme, we packaged L-ASNases into microcapsules, thus enhancing stability and potentially preventing exposure of the enzyme to the immune system. We applied the Layer-by-Layer (LbL) strategy of biocompatible microcapsule formation, using calcium carbonate particles as core templates for protein adsorption, which were coated with poly-L-arginine and dextran sulfate polymers. Our work suggests that microcarriers in combination with enzyme engineering will set the basis for novel ways to treat blood cancers.

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