Meet the scientist

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Dr. Jeremias’ research aim is to improve the treatment of children and adults suffering acute leukemia, both the lymphoblastic (ALL) as well as the myeloid (AML) subtype. By generating genetically engineered patient-derived xenograft (PDX) leukemia models, she collaborates with partners worldwide to identify and validate novel treatment options for these patients. Her focus is on eliminating leukemia stem cells to prevent disease relapse and improve outcome.

Patient-derived xenograft (PDX) cancer models are rapidly replacing long-established traditional cell lines as preferred models for conducting basic and translational preclinical research. PDX models are established by transferring patient tumor fragments into immunodeficient mice. This allows tumor growth and subsequent transplantation into secondary recipient mice. PDXs maintain the cellular and histopathological structures of the original tumors as their growth continues to depend on interaction with the tumor environment of a living organism. They are a robust and low-throughput way to model human tumors in vivo.

Convinced that PDX models could be used to gain functional insights into acute leukemias, Dr. Irmela Jeremias has been evaluating the relevance of specific tumor alterations using gene silencing techniques in PDX models. “We wanted to find dependencies and drug targets in acute leukemias to improve the therapy that patients get in order to improve their prognosis outcome,” explained Dr. Jeremias. “Our approach allows us to molecularly mimic in a preclinical in vivo mouse model what will happen when a patient takes a drug targeted against a particular gene protein.”

Dr. Jeremias’ interest in PDX models stemmed from the inherent shortcomings of using in vitro models to study acute leukemias. “We only have a few cell lines that exist, and there is a major difference between cell lines and patient cells,” she explained. “For example, the P53 protein, or the TP53 gene, is seldomly mutated in primary acute leukemia samples from patients but is very often mutated in cell lines. This means the cell lines do not give us the exact models to study in order to translate our results into the clinics.” Instead, she turned to PDX models which she believes better recapitulate the complexity of human tumors in a reliable and reproducible way.
Generating genetically engineered PDX leukemia models

To develop an inducible knockdown system in PDX acute leukemia cells, Dr. Jeremias and colleagues first transplanted primary tumor cells from patients with acute leukemias into severely immuno-compromised mice. The resulting PDX cells were then genetically engineered with lentiviruses, first to constitutively express a Tamoxifen (TAM)-inducible variant of Cre recombinase, Cre-ER\textsuperscript{T2}, together with mCherry and luciferase, and second to express inducible knockdown vectors. “We wanted to have an inducible system that could alter mRNA expression to find genes with an essential function in established tumors,” she explained. Mice were then transplanted with a 1:1 mixture of PDX cells from the same patient expressing one of two RNAi vectors: one encoding a small hairpin (sh) RNA targeting luciferase (shCTRL), the other encoding an shRNA targeting a gene of interest (shGOI). In mice with established leukemias, TAM could then be administered to induce Cre-ER\textsuperscript{T2}-mediated recombination, mimicking the treatment of patients with pre-existing tumors.

Once the team had established this technique, they set out to perform gene knockdown of specific genes to assess heir function in the tumor. First, they analyzed the response of PDX samples to shRNA-mediated inhibition of MCL1. “MCL1 is a member of the BCL-2 family, and it is known to be overexpressed in many tumors,” said Dr. Jeremias. She added that although MCL1 is a well-known target, not all leukemia seems to respond to MCL1 inhibition. They studied PDX models from three patients with acute leukemia and found that MCL1 silencing induced cell death in just one of the three PDX models, suggestive of interpatient variability. Notably, these findings also correlated with pharmacological MCL1 inhibition in patient tumors. When they treated the models, an MCL-1 inhibitor reduced tumor burden only in the model which showed an effect of MCL-1 knockdown. The findings demonstrate how this approach can distinguish between individual tumors to select patients who might profit from therapies targeting a gene of interest.

In another set of experiments, the researchers targeted the fusion oncogene MLL-AF4, which is present in 80% of infant B-precursor acute lymphoblastic leukemias (ALL) and is associated with poor prognosis. They generated shRNA targeting the fusion mRNA product and found that inducible knockdown of MLL-AF4 significantly reduced ALL cells in the PDX model. This suggests a tumor-maintaining role of MLL-AF4 in patient-derived leukemias growing in vivo. “The strength of this model is that if the mRNA of the fusion gene is important for PDX models in vivo, the likelihood is very high that there will be patients where this is also the case,” enthused Dr. Jeremias.

Challenges of PDX models of acute leukemias

While PDX models are heralded for their ability to maintain the cellular and histopathological structures of the original tumors, Dr. Jeremias cautions that from a precision medicine perspective, they are not a one-to-one predictive model for the patient. “This is the dream, but I don’t think it will come true due to clone heterogeneity,” she said. “In acute myeloid leukemia, for example, if you take the tumor and put it into 50 mice, you will have different clone mixtures engrafting in different mice. There are the more aggressive clones, which are more like relapse cells. But if you then take just one of those mice and go on with your studies, you are never sure whether this particular PDX model contains the clone which limits the prognosis of your patient.” Instead, Dr. Jeremias sees the strength of PDX models as a proof-of-concept, providing important physiologically relevant information on signaling networks, pathways, principles, and treatment options. “If we can prove something to be true in several of the PDX models then we can say that there is a high likelihood of it being true in a number of patient cells.”

She also notes that in vivo surroundings have a major impact on the effects of therapy, with pharmacodynamics and pharmacokinetics varying considerably in the mouse compared to humans. When compared to solid tumors, modeling leukemia has the advantage of a suspension tumor that distributes well in the body of mice. “Although the mouse environment is not the human environment, it seems to have important similarities because the tumor cells home to exactly the same places as they would in humans,” she said.

Looking forward, Dr. Jeremias plans to continue using PDX models as a proof-of-concept to understand essential dependencies required for cell growth and survival.
Such dependencies represent attractive therapeutic targets for reducing tumor burden, and the hope is to not only work with known targets but also discover new ones. She also suggests the models could be used for quality control of biomarkers. “We have established large cohorts of PDX models with different genetics, expression profiles, and risk groups,” she explains. “So, if you would like to know whether your biomarker is predictive for response, you could take 20, 30, or more PDX models and try it out.”

Despite the technical challenges of developing these models, Dr. Jeremias is convinced that for acute leukemias, they are currently the closest model system to patients. “We have the essential genes, pathways, and networks present in the PDX cells, so this technique allows us to molecularly mimic in a preclinical in vivo mouse model what will happen when a patient takes a drug targeted against a specific gene. Together with the subsequent preclinical treatment trials, I think this is the closest to the patient that we can get.”

More about the scientist

Dr. Jeremias has been a Medical Doctor at Dr. von Haunersches Kinderspital of LMU Munich since 1993. She started as a Resident, later was an Attending Physician in Intensive Care and board-certified Pediatrician, and currently has a focus on Molecular Tumor Board. After her PostDoc at the German Cancer Research Center, she started her own lab in Munich in 1998. In 2005, she started with a Junior Research group at Helmholtz Munich, where she still works today, as tenured group leader and later as head of department. She is a part of the German Cancer Research Center, several Collaborative Research Centers, and Leukemia Study Groups.