MOLECULAR MECHANISM OF NON-GENETIC ADAPTATION TO BTK INHIBITOR THERAPY IN CLL

Ondrisova L.[1,2], Seda V.[1,2], Hlavac K.[1,2], Hoferkova E.[1,2], Pavelkova P.[1,2], Kostalova L.[1,2], Filip D.[1,2], Faria Zeni P.[2], Panovska A.[1], Plevova K.[1], Pospisilova S.[1], Simkovic M.[3], Vrbacky F.[3], Lysak D.[4], Davids M.S.[5], Martin-Subero J.I.[6], Brown J. R.[5], Doubek M.[1], Forconi F.[7], Mayer J.[1], Mraz M.[1,2]

[1] *FN Brno, ČR*

[2] *CEITEC MU, ČR*

[3] *FN Hradec Králové, ČR*

[4] *FN Plzeň, ČR*

[5] *Dana-Farber Cancer Institute, Boston, USA*

[6] *University of Barcelona, Spain*

[7] *University of Southampton, UK*

Genetic mechanisms of resistance to BTK inhibitors in CLL have been described. However, it remains unknown whether non-genetic adaptation to BTK inhibitors might exist. We focused on the possible role of the Akt pathway since, in mouse models, PI3K-Akt activation rescues the apoptosis induced by BCR deletion in mature B cells (Srinivasan et al. Cell, 2009). We show that in ~70% of CLL cases, ibrutinib increases Akt activity ($pAkt^{S473}$) above pre-therapy levels (31 patients with 87 samples; $P < 0.005$). pAkt was also restored in ibrutinib treated MEC1 cells ($P < 0.05$). Importantly, CLL cells obtained during ibrutinib therapy *in vivo* were highly sensitive (90% apoptosis) to Akt inhibitor MK2206. RNA profiling of paired CLL samples obtained before and during ibrutinib (N = 22) or single-agent idelalisib therapy (N = 18) identified 16 differentially expressed mRNAs (with both drugs) involved in the PI3K-Akt pathway. Rictor induction was particularly notable since it is an essential assembly protein for mTORC2, which is known to phosphorylate Akt directly on S473 (Sarbassov et al. 2005). Analysis of samples obtained during therapy and genome-editing experiments in MEC1 cells revealed that transcription factor FoxO1 is directly responsible for Rictor/pAkt activation during ibrutinib treatment, and FOXO1 is required for adaptation to BTK inhibitors. FoxO1 inhibitor (AS1842856) decreased pAkt levels, induced apoptosis alone (~40% CLL cell killing) or more potently in combination with ibrutinib (~60% apoptosis; N = 7). In summary we describe the first non-genetic adaptation to targeted therapy with BCR inhibitors in CLL.

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POINT-OF-CARE NON-VIRAL PRODUCTION OF CD19 CHIMERIC ANTIGEN RECEPTOR-T CELLS WITH ENHANCED TSCM IMMUNOPHENOTYPE FOR THERAPY OF REFRACTORY B-CELL MALIGNANCIES – PRELIMINARY RESULTS OF PHASE I CLINICAL TRIAL

Otáhal P., Vydra J., Štach M., Šťastná Marková M., Válková V., Šálek C., Petráčková M., Rychlá J., Kaštánková I., Pytlík R., Lesný P., Cetkovský P.

Institute of Hematology and Blood Transfusion, Prague

Background: The efficacy of CAR-T therapy is partially influenced by immunophenotype of the manufactured CAR-T cells. Products containing higher percentage of less-differentiated, T stem-cell memory subsets provide longer persisting therapeutic effect. We developed an alternative production method of non-viral gene transfer by electroporating non-activated T cells with piggyBac transposon DNA, followed by expansion in the presence of IL-21 cytokine to increase the prevalence of CAR-T cells with stem-cell memory phenotype. The product, designated UHKT CAR19, expresses a CAR containing a unique CD19-specific scFv and, 4-1BB-zeta intracellular signaling domain.

Aims: To develop the point-of-care production pipeline of CAR-T manufacturing and initiate a clinical trial with CD19-specific CAR-T in patients with B-ALL/B-NHL.

Methods: A phase I study (NCT05054257) was initiated to evaluate the safety and efficacy of autologous UHKT CAR19 in adult patients with relapsed or refractory B-ALL or B-NHL who were not eligible for approved commercial CAR-T therapy. This phase I trial uses a dose-escalating scheme, where all patients receive 25 $mg/m^2/d$ of fludarabine for 5 days and 500 mg/m 2/d of cyclophosphamide for 2 days followed by a single infusion of UHKT CAR19. A group of patients (N = 15) treated with tisa-cel as a standard-of-care was used to compare the properties of the manufactured product, such as the immunophenotype, expansion after administration to patients, and preliminary clinical response in the ongoing clinical trial.

Results: In the pre-clinical studies, the UHKT CAR19 T cells showed cytotoxic effect ex vivo on CD19 positive tumor cell lines, and efficiently inhibited tumor growth and demonstrated safe toxicity profile in NSG mouse models. CAR gene integration sites were determined by targeted locus amplification method showing equal distribution throughout the genome without preference for specific sites. The UHKT CAR19 displayed a higher percentage of stem-cell memory CAR-T cells compared to with tisa-cel, in both CD4+ and CD8+ CAR+ T cells. The UHKT CAR19 had elevated percentage of CD45RA+CD62L+ (43 vs. 12%; $P = 0.0007$; 61 vs. 19%; $P = 0.0012$),