JNJ-87890387, A Novel ENPP3 Bispecific T-cell Redirector (ENPP3xCD3) with Tumor Selectivity Through Targeting Apical Surface Antigens

LB122

Smruthi Vijayaraghavan, Pankaj Seth, Annmarie Winkis, Kristen Chevalier, Alexa Marthaler, Katrin Sproesser, Vince Torti, Nirav Shah, Eilyn Lacy, Anna-Lena Frisk, Heather Deutsch, Gerald Chu, Michael Sharp, Jonathan Pabalan, Cholpon Tilegenova, Sitilia Rencheli, Katherine Thornton, Bethany Mattson, Ken Tian, Jacqueline Kinyamu-Akunda, Josh Lauring, Laurie Lenox, Wan Cheung Cheung, Sheri Moores, Sylvie Laquerre, Yusri Elsayed.

Janssen Research & Development, LLC, a Johnson & Johnson Company, Spring House, PA

Abstract

Clinical success of T-cell redirection therapy for solid tumors has been limited partly due to a lack of cancer-specific targets, leading to on-target off-tumor toxicity at sub-efficacious doses. ENPP3 (ectonucleotide pyrophosphatase/phosphodiesterase family member 3) is a transmembrane protein identified using an artificial intelligence machine learning algorithm. ENPP3 is characterized by apically restricted expression in most normal tissues and elevated, depolarized expression in several solid tumors, thus potentially providing tumor selectivity. ENPP3 expression exhibits a high prevalence in several solid tumors including renal cell carcinoma (RCC) – clear cell (93%) and papillary (78%), lung adenocarcinoma (50%), endometrioid uterine (53%) and ovarian (47%) cancers, and colorectal carcinoma (51%).

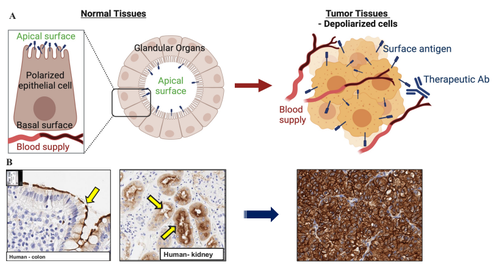
JNJ-87890387 is a fully human bispecific antibody with high affinity and specific binding to ENPP3 on tumor cells and lower affinity binding to cluster of differentiation (CD)3 on T-cells. In vitro, JNJ-87890387 demonstrated potent (pM) T-cell activation and cytotoxicity against tumor cell lines with different levels of endogenous ENPP3 membrane expression, and absence of killing against cells lacking ENPP3 expression, confirming JNJ-87890387 specificity. In vivo, JNJ-87890387 demonstrated ENPP3-expression-dependent potent anti-tumor activity in multiple cell line-derived and patient tumor-derived RCC and HCC xenograft models with complete tumor regressions, and evidence of dose-dependent CD4+ and CD8+ T cell infiltration into the tumors. Safety assessment in the cynomolgus monkey with a tool molecule using the therapeutic ENPP3 binding arm and a cynomolgus CD3 cross-reactive arm provided data that support further clinical evaluation.

In summary, these data support JNJ-87890387's clinical development with an ongoing first-in-human Phase I study to evaluate the safety and preliminary anti-tumor activity in advanced-stage solid tumors with a high prevalence of ENPP3 expression.

Background

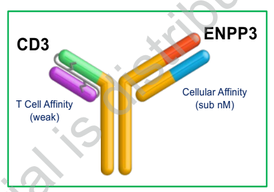
- ENPP3 was identified through AI-image-based target mining approach with apically restricted expression in most normal tissues and elevated disorganized expression in several solid tumors.
- ENPP3 is a 150kDa single-pass Type II transmembrane protein with ATPase and pyrophosphatase activities involved in the hydrolysis of extracellular nucleotides. Its biological role in cancer is not well understood.
- ENPP3 is a unique tumor-targeting antigen with high tumor expression and apically-restricted normal expression, making it a highly attractive cancer target for CD3 redirected T-cell killing.
- JNJ-87890387 is a fully human bispecific antibody with higher-affinity ENPP3 binding paired with lower-affinity CD3 binding.

Figure 1. Apical surface target – Novel class of polarization-based tumor-specific antigens



(A) Cartoon depicting the polarized expression of ENPP3 on the apical surface of normal glandular organs compared to the depolarized expression in tumor tissues. (B) Representative IHC images showing ENPP3 apical expression in normal healthy tissues and depolarized expression in clear cell renal cancer tissues (right).

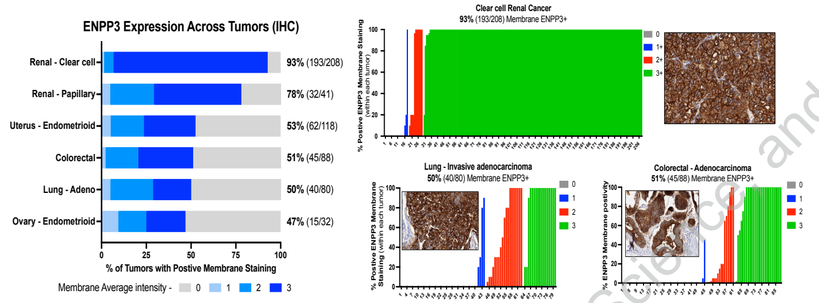
Figure 2. JNJ-87890387 molecular design



Cartoon depicting the molecular design of the lead ENPP3xCD3 bispecific antibody.

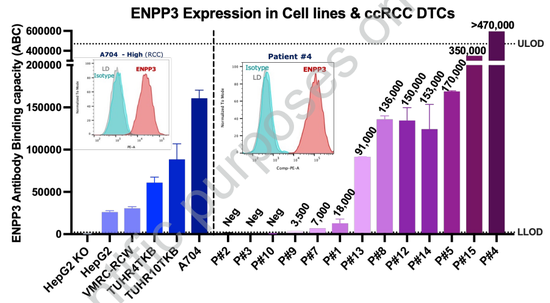
Results

Figure 3: Solid tumor expression of ENPP3 evaluated by IHC



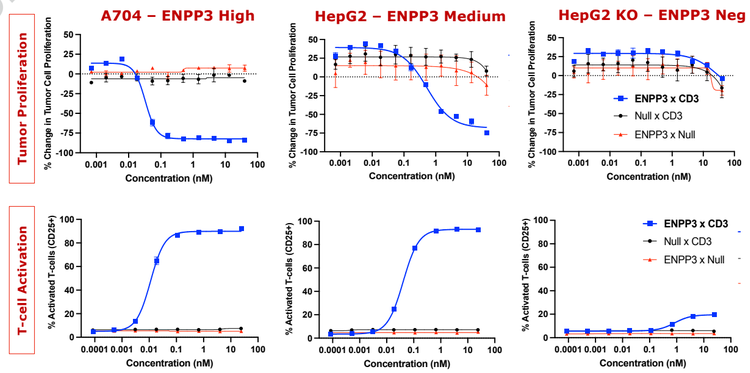
IHC-based evaluation of ENPP3 expression on TMAs from multiple solid tumors. Percentage of samples scoring membrane-positive with intensity of ENPP3 membrane staining is depicted as 0 (negative), 1+ (weak), 2+ (moderate), and 3+ (strong). IHC-based evaluation of ENPP3 membrane expression on ccRCC, lung and colorectal tumor TMAs. Graphs show the distribution of % ENPP3 positivity as well as staining intensity. Representative images showing IHC positivity (Magnification: 20X).

Figure 4: Quantification of ENPP3 expression in cell lines and RCC tumors



Flow-cytometry-based membrane ENPP3 detection and receptor occupancy measured on a panel of endogenous cancer cell lines and in 13 clear cell renal cell carcinoma (ccRCC) dissociated tumor (DTC). Representative histograms showing ENPP3 expression in A704 and one of the positive ccRCC DTC.

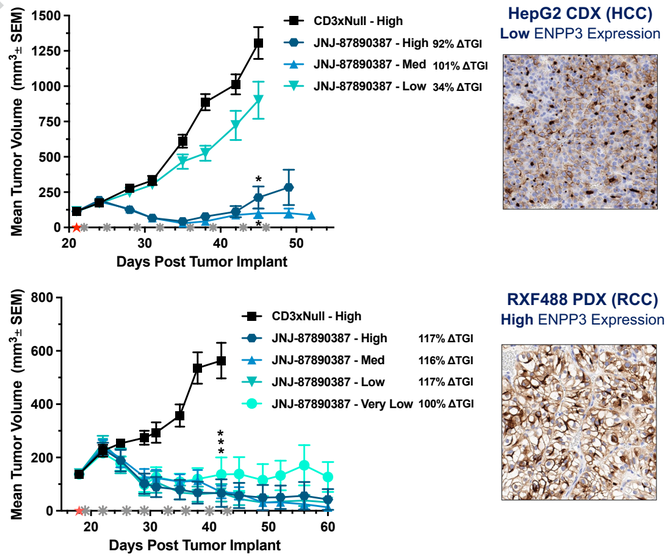
Figure 5: ENPP3xCD3 bispecific antibody induced cell killing and T-cell activation in ENPP3-expressing tumor cell lines



Incucyte-based assessment of tumor cell killing and flow cytometry-based T-cell activation (CD25+ T cells) was measured upon treatment with ENPP3xCD3, ENPP3xNull, and NullxCD3 antibodies in the presence of isolated T cells on a panel of cell lines. Error bars are SEM.

Results

Figure 6: ENPP3xCD3 bispecific antibody demonstrated robust anti-tumor efficacy in ENPP3+ CDX and PDX models



NSG mice bearing established HepG2 and RXF488 xenografts (engrafted with human T cells indicated by red stars) were dosed with ENPP3xCD3 or NullxCD3 antibodies (indicated by gray stars) and changes in group tumor volumes are graphed as mean ± SEM. Black stars indicate statistically significant ΔTGI. IHC shows ENPP3 expression measured on ex vivo tumors.

Conclusion

- High prevalence of ENPP3 was detected in several solid tumors including renal cell carcinoma (RCC) – clear cell (93%) and papillary (78%), lung adenocarcinoma (50%), endometrioid uterine (53%) and ovarian (47%) cancers, and colorectal carcinoma (51%).
- JNJ-87890387 is a human BsAb comprising of a higher-affinity ENPP3 specific binding arm paired with a lower-affinity CD3 binding arm. JNJ-87890387 demonstrated a favorable biophysical profile and good conformational stability.
- Preclinical studies demonstrated that JNJ-87890387 induced potent ENPP3 - specific *in vitro* T-cell activation, T-cell-mediated cytotoxicity, and pro-inflammatory cytokine release across multiple ENPP3 expressing tumor cell lines.
- *In vivo* studies in CDX and PDX models with low and high ENPP3 expression, demonstrated robust anti-tumor efficacy, with complete regressions.
- An IND-enabling cynomolgus monkey study with an ENPP3xCD3 tool BsAb identified monitorable and manageable findings.
- In summary, these data support JNJ-87890387's clinical development with an ongoing first-in-human Phase I study (NCT06178614) to evaluate the safety and preliminary anti-tumor activity in advanced-stage solid tumors with a high prevalence of ENPP3 expression.

Presenter's Contact:
Smruthi Vijayaraghavan
svijayar@its.jnj.com