

# Tumor Infiltration and Cytokine Biomarkers of Prostate Stem Cell Antigen (PSCA)-Directed GoCAR-T® Cells in Patients with Advanced Pancreatic Tumors

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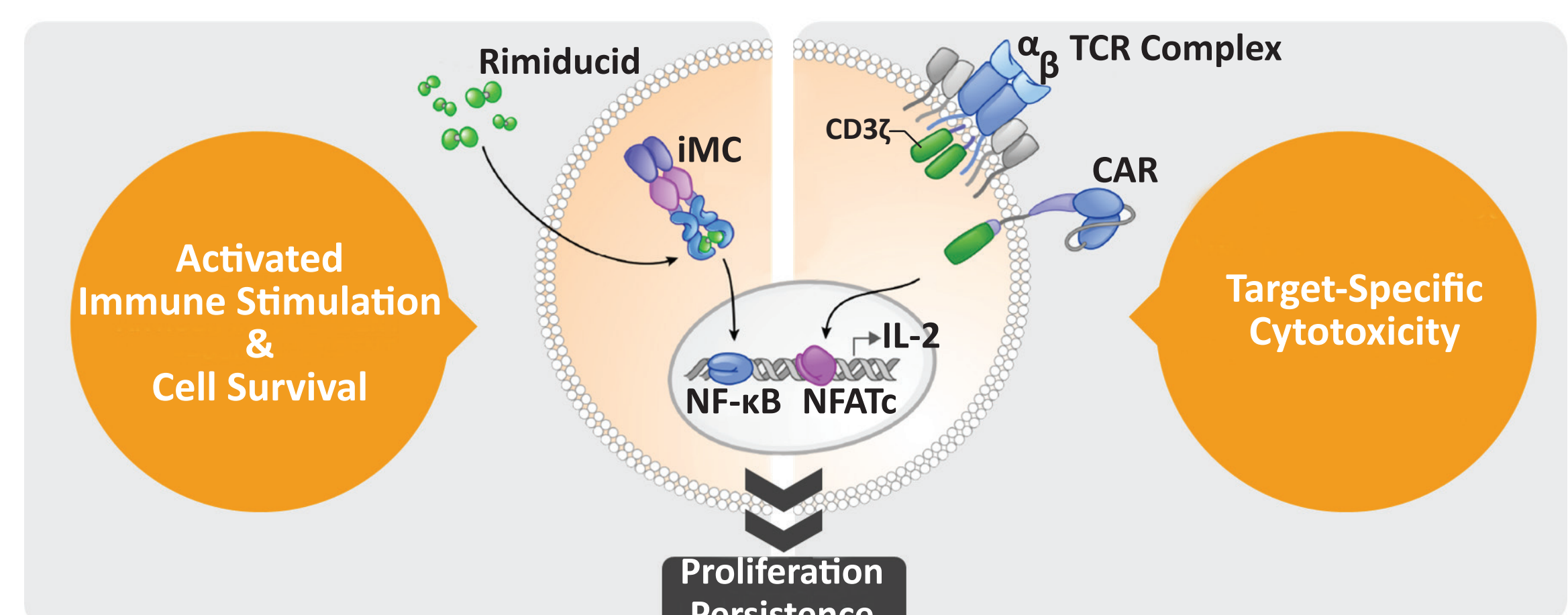
## BACKGROUND

Prostate Stem Cell Antigen (PSCA) is a cell surface protein overexpressed in approximately 50-80% of pancreatic cancers.<sup>1-3</sup> BPX-601 is an autologous GoCAR-T cell therapy engineered to express a PSCA-CD3 $\zeta$  CAR and the inducible MyD88/CD40 (IMC) coactivation domain. Activated by the ligand rimiducid (Rim), IMC is designed to boost CAR-T performance in solid tumors. The safety and activity of BPX-601 activated with Rim in PSCA+ metastatic pancreatic cancer is currently being assessed in a Phase 1/2 clinical trial, BP-012 (NCT02744287).

Phase 1 of BP-012 is a 3+3 dose escalation of BPX-601 (1.25-5  $\times 10^6$  cells/kg) administered on Day 0 with a single, fixed-dose of Rim (0.4 mg/kg) on Day 7 in subjects with previously treated PSCA+ metastatic pancreatic cancer. A total of 18 subjects have been enrolled and treated with BPX-601. Fourteen subjects received rimiducid on Day 7 following cell infusion. Cohorts 0 (n=3), 3 (n=3), 4 (n=3) and 5A (n=4) received single dose of Cy lymphodepletion (LD). Cohort 5B (n=5) subjects received Flu/Cy LD over 3 days followed by BPX-601 (5  $\times 10^6$  cells/kg) and Rim (0.4 mg/kg) on Day 7. The current study presents a brief biomarker summary analysis from all cohorts (n=18) of BPX-601 cell proliferation and persistence as well as the production of several CAR-T-associated cytokines (IFN- $\gamma$ , IL-6, IP-10, and GM-CSF). An in-depth biomarker analysis of Cohort 5B (n=5) was performed, including serum cytokine profiles (n=5), tumor infiltration by GoCAR-T cells and differential gene expression from available tumor biopsies collected (n=3).

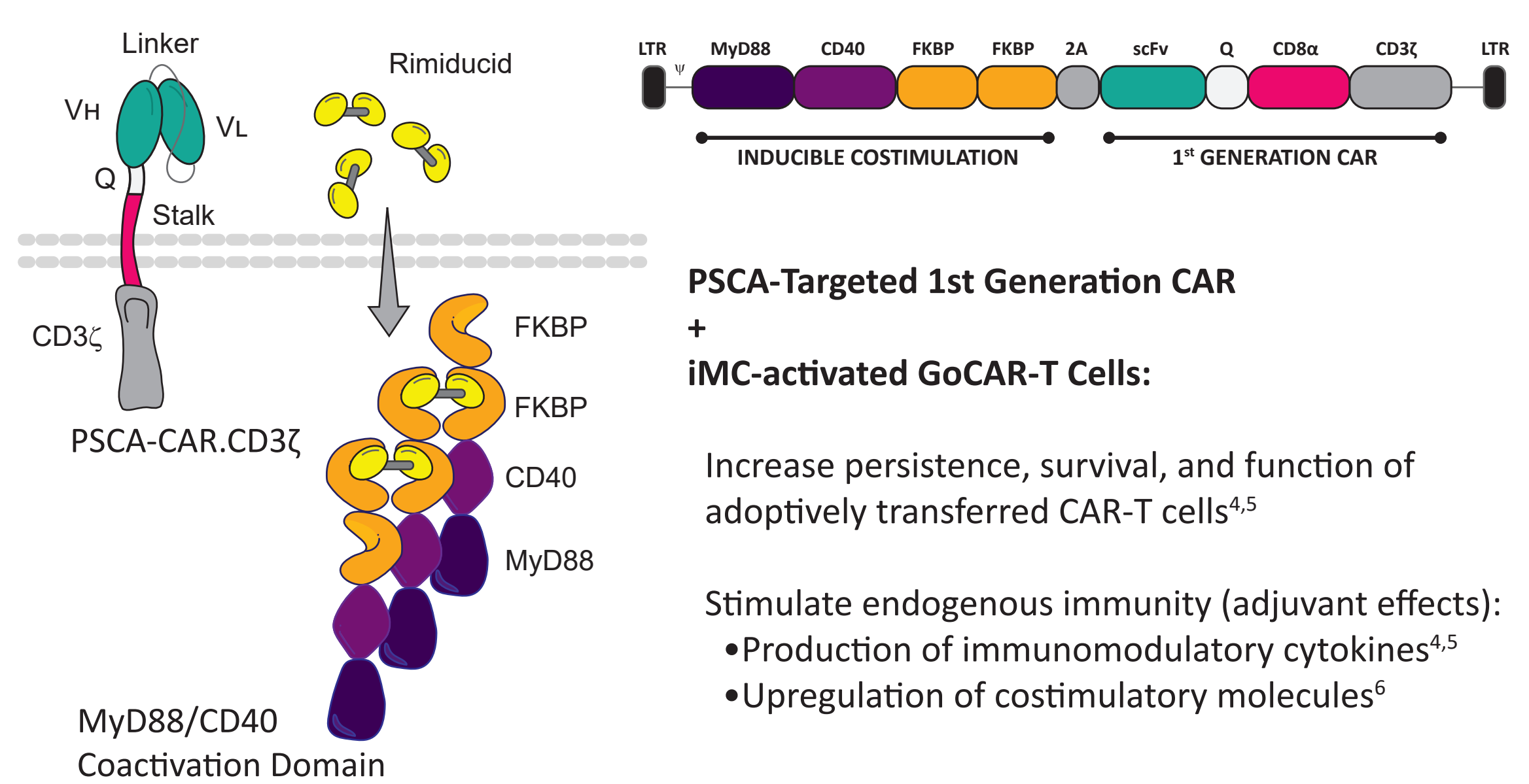
## GoCAR-T PLATFORM

### CAR-T Enhanced by IMC Co-activation



The GoCAR-T platform incorporates the IMC coactivator into CAR-T cells to provide powerful activation of immune pathways, downstream of MyD88 (innate) and CD40 (adaptive) signaling. Activation of IMC with rimiducid promotes cell proliferation, persistence, secretion of immunomodulatory cytokines, and resistance to T cell exhaustion.<sup>4,5</sup>

## BPX-601 GoCAR-T DESIGN



## TARGET ANTIGEN: PSCA

- Small, GPI-anchored cell-surface protein of the Thy-1/Ly-6 family
- Expression observed in 50-80% of pancreatic ductal adenocarcinomas<sup>1-3</sup>
- Low basal expression on normal prostate epithelium, urinary bladder, kidney, esophagus, stomach, and placenta<sup>2</sup>
- Low toxicity profile with PSCA-targeted antibodies in prostate and pancreatic cancer<sup>7,8</sup>

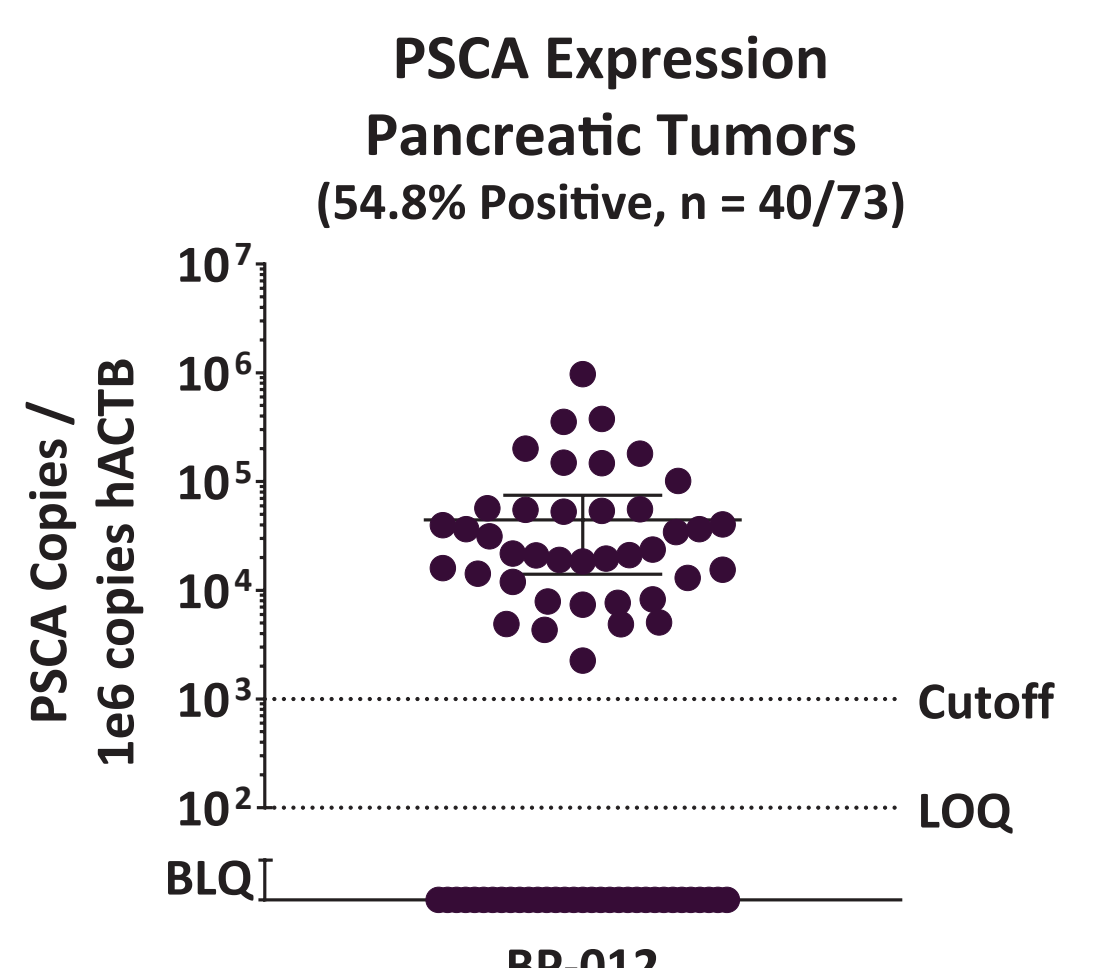
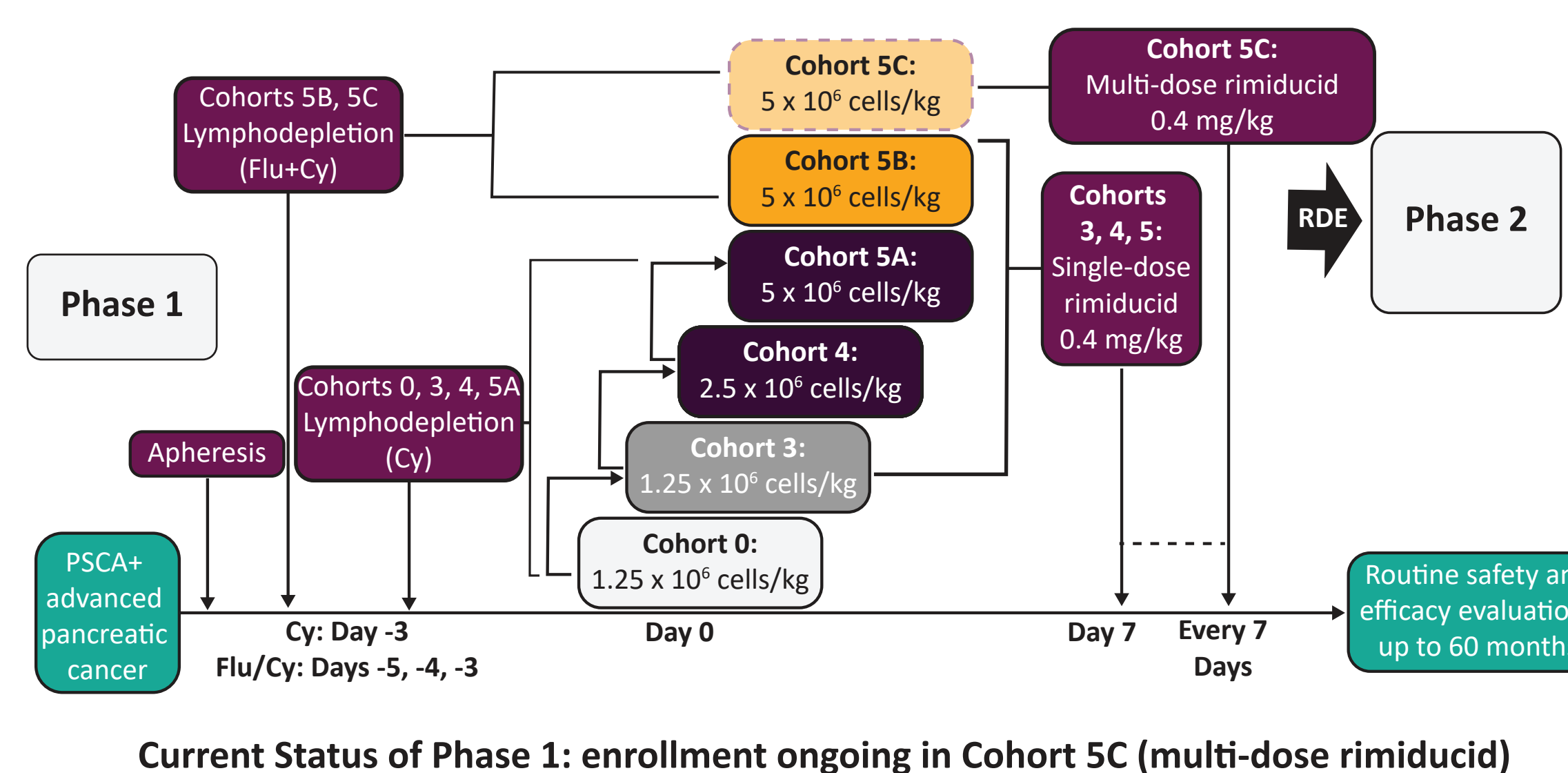


Figure 1. PSCA expression measured by qPCR in BP-012 pancreatic tumor screening samples. Data as of October 15, 2019.

## REFERENCES

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## BP-012 STUDY DESIGN



Current Status of Phase 1: enrollment ongoing in Cohort 5C (multi-dose rimiducid)

Cohort 0: Lead-in safety cohort with cells only and no rimiducid  
Cohorts 3-5B: Increasing cell doses based on a 3+3 dose escalation design followed by fixed dose of rimiducid on Day 7  
Cohort 5C: Maximum cell dose (5  $\times 10^6$  cells/kg) followed by rimiducid on Day 7 and every 7 days thereafter until unacceptable toxicity or progression of disease.

Cy, cyclophosphamide; Flu, fludarabine; PSCA, prostate stem cell antigen; RDE, recommended dose for expansion

## PATIENTS

Table 1. BP-012 Patient Demographics and Clinical Characteristics

Cohort	LD Regimen	BPX-601 Dose (10 <sup>6</sup> cells/kg)	Rim (Y/N)	Patient	Age/Sex	# Prior Systemic Anti-Cancer Therapies	Prior Anti-Cancer Immunotherapy	History of Surgical Resection	PSCA (copies)	Best Response <sup>a</sup>
0	Cy	1.25	N	0-1	50/F	1	N	N	5,071	N/E <sup>b</sup>
			N	0-2	58/F	3	N	Y	377,444	N/E <sup>b</sup>
			N	0-3	65/F	5	N	N	15,494	N/E <sup>b</sup>
3	Cy	1.25	Y	3-1	59/F	2	Investigational DC vaccine (WT1, mesothelin)	N	34,342	SD
			Y	3-2	70/M	1	N	N	7,367	SD
			Y	3-3	58/F	1	N	N	31,429	PD
			Y	4-1	71/F	2	N	N	7,686	SD
4	Cy	2.5	Y	4-2	65/M	2	Anakirra	Y	8,243	PD
			Y	4-3	60/F	3	N	N	354,730	SD
			Y	5A-1	64/M	1	N	N	19,533	N/A <sup>c</sup>
			Y	5A-2	61/M	2	Anakirra	Y	14,238	PD
5A	Cy	5.0	N	5A-3	59/M	5	Pembro	N	969,094	N/A <sup>c</sup>
			Y	5A-4	55/M	2	N	N	52,797	SD
			Y	5B-1	77/M	4	N	Y	13,930	SD
			Y	5B-2	56/M	4	N	Y	201,277	PD
			Y	5B-3	72/M	1	N	N	21,099	SD
			Y	5B-4	68/F	1	N	N	54,877	PD
			Y	5B-5	58/M	1	N	N	149,110	SD

<sup>a</sup>Cy dose = 1 g/m<sup>2</sup> Day -3; Flu/Cy dose = Cy 500 mg/m<sup>2</sup> + Flu 30 mg/m<sup>2</sup> Days -5, -4, and -3

<sup>b</sup>Prior history of systemic therapy regardless of intent

<sup>c</sup>History of pancreatoduodenectomy

<sup>d</sup>Response Criteria as measured by RECIST 1.1

Cohort 0 subjects not evaluable per RECIST 1.1; Subject 5A-1 NED at baseline; Subject 5A-3 off study prior to first scan due to AE associated with progressive disease

Safety data for Cohorts 0-5B reported in Becerra et al., Annual Meeting 2019, American Society of Clinical Oncology

Cy, cyclophosphamide; Flu, fludarabine; N/A, not available; N/E, not evaluable; NED, no evidence of disease; Pembro, pembrolizumab; PD, progressive disease; PSCA, prostate stem cell antigen; Rim, rimiducid; SD, stable disease.

## BIOMARKER SAMPLING TIMELINE

BP-012 Cohort 5B Schedule Shown

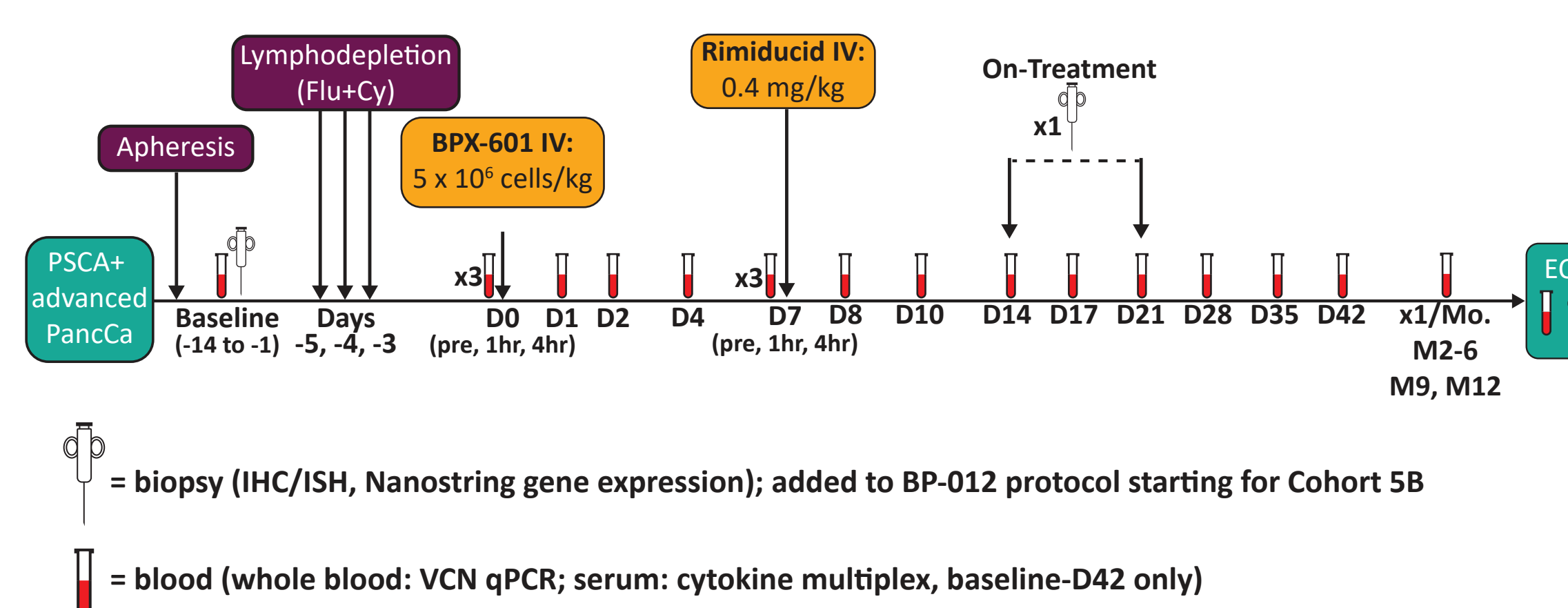


Table 2. Biomarker Sampling by Cohort

Cohort	Cohort 0 1.25 $\times 10^6$ cells/kg (Cy)	Cohort 3 1.25 $\times 10^6$ cells/kg +Rim (Cy)	Cohort 4 2.5 $\times 10^6$ cells/kg +Rim (Cy)	Cohort 5A 5 $\times 10^6$ cells/kg +Rim (Cy)	Cohort 5B 5 $\times 10^6$ cells/kg +Rim (Flu/Cy)
# of Patients	n=3	n=3	n=3	n=4	n=5
BPX-601 Tracking (Vector Copy Number)	✓	✓	✓	✓	✓
Serum Cytokines (Multiplex)	✓	✓	✓	✓	✓
Tumor Biopsy* (IHC, ISH, NanoString)	NA	NA	NA	NA	✓ (n=3/5)

\*Baseline and on-treatment tumor biopsies added to BP-012 clinical protocol starting for Cohort 5B

## COHORTS 0-5B BIOMARKER OVERVIEW

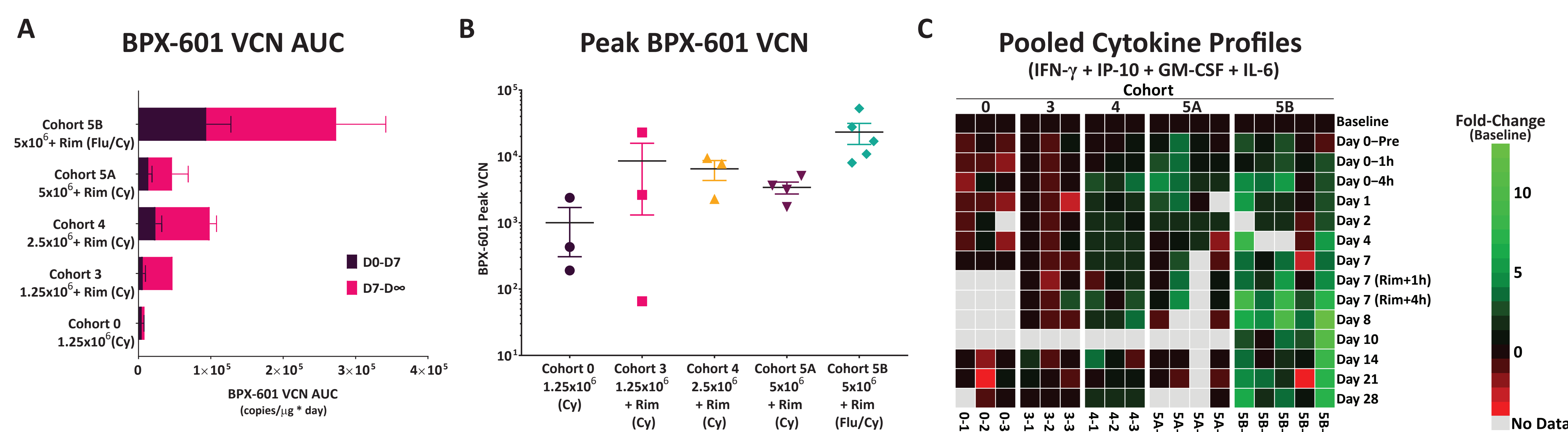
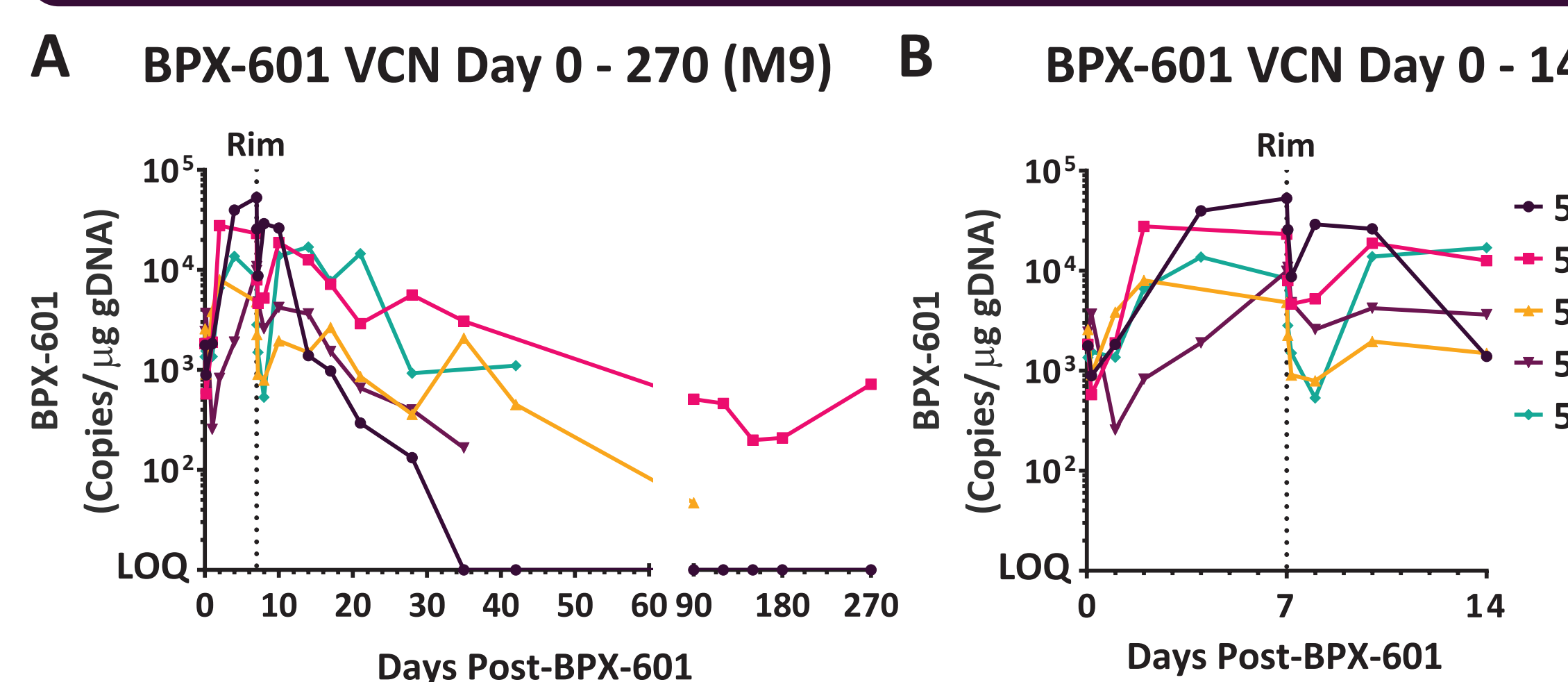


Figure 2. BPX-601 vector copy number (VCN) was quantitated by qPCR from isolated PBMC samples. (A) Area under the curve (AUC) for BPX-601 VCN over time was calculated by the linear trapezoidal method using PK Solver. Patients without an evaluable terminal elimination phase were omitted from the D7-Dinfinity dataset. (B) Peak BPX-601 VCN observed. (C) Serum cytokines were measured in patient samples using a multiplex Luminex assay. Each box shows weighted-mean fold-change serum concentration (pg/ml) from baseline for CAR-T-associated cytokines, pooling IFN- $\gamma$ , IP-10, GM-CSF, and IL-6 together.

## COHORT 5B BIOMARKER RESULTS

## PROLIFERATION & PERSISTENCE



\*Patient 5B-3 received corticosteroids Day 8 for grade 2 encephalopathy.

\*Patient 5B-5 received tocilizumab Day 8 for grade 2 encephalopathy.

Figure 3. (A) Persistence of BPX-601 cells in cohort 5B patients was evaluated by vector copy number (VCN) analysis. BPX-601 cells expanded in all patients (n=5) and persisted up to 9 months. Four of 5 (80%) patients have measure BPX-601 VCN at last time point available. (B) Rapid reduction and rebound in BPX-601 VCN was observed in all patients (n=5) following rimiducid infusion.

## ACTIVATED T CELL REDISTRIBUTION

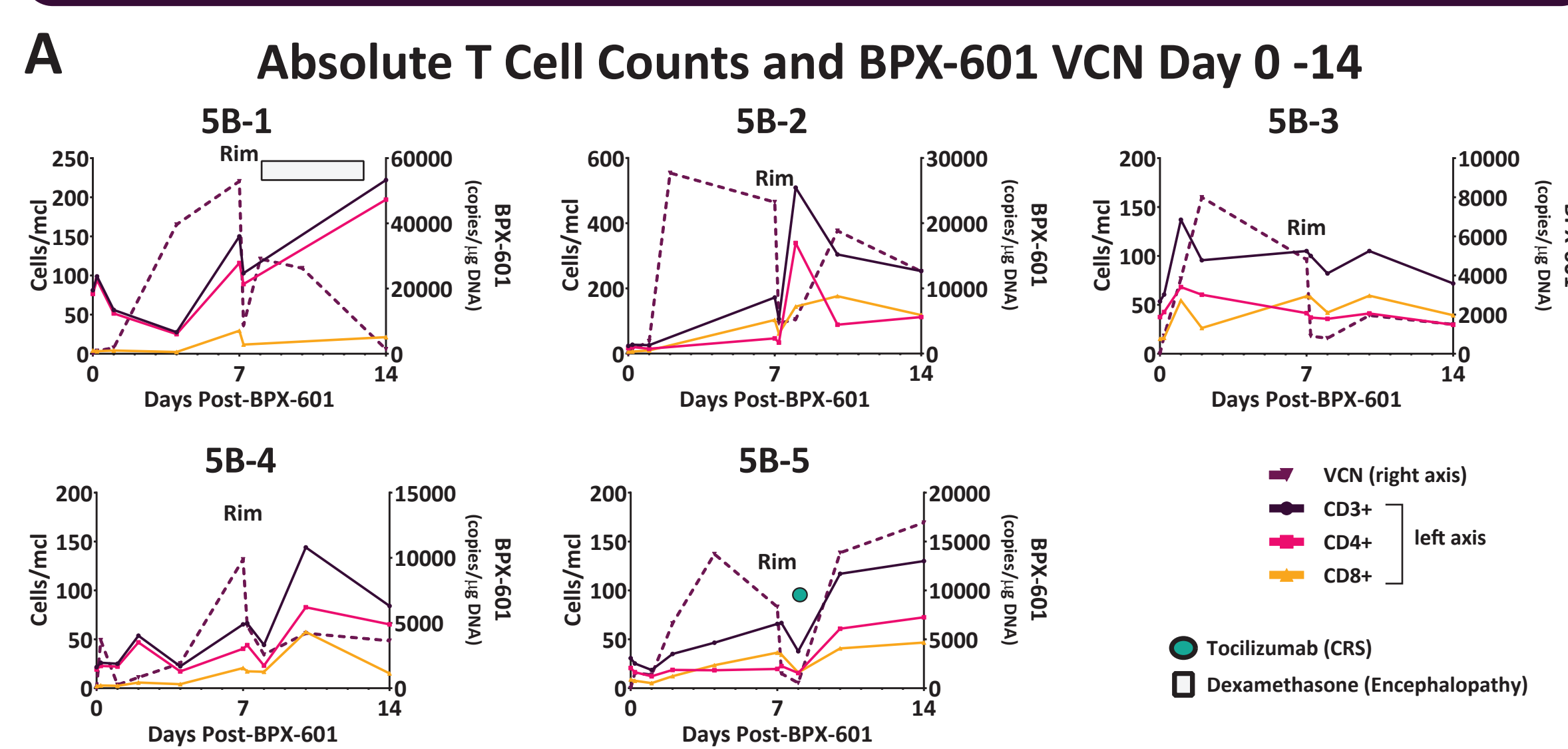


Figure 4. (A) A rapid, transient reduction and rebound in the absolute number of total T cells (CD3) and CD4+ and CD8+ T cell subsets in cohort 5B patients (n=5) was observed following rimiducid infusion, concurrent with the decrease in BPX-601 VCN. This suggests redistribution of activated T cells from the blood stream potentially by vascular adhesion associated with T cell transmigration.

## GoCAR-T ACTIVATION INCREASED IMMUNOMODULATORY CYTOKINES

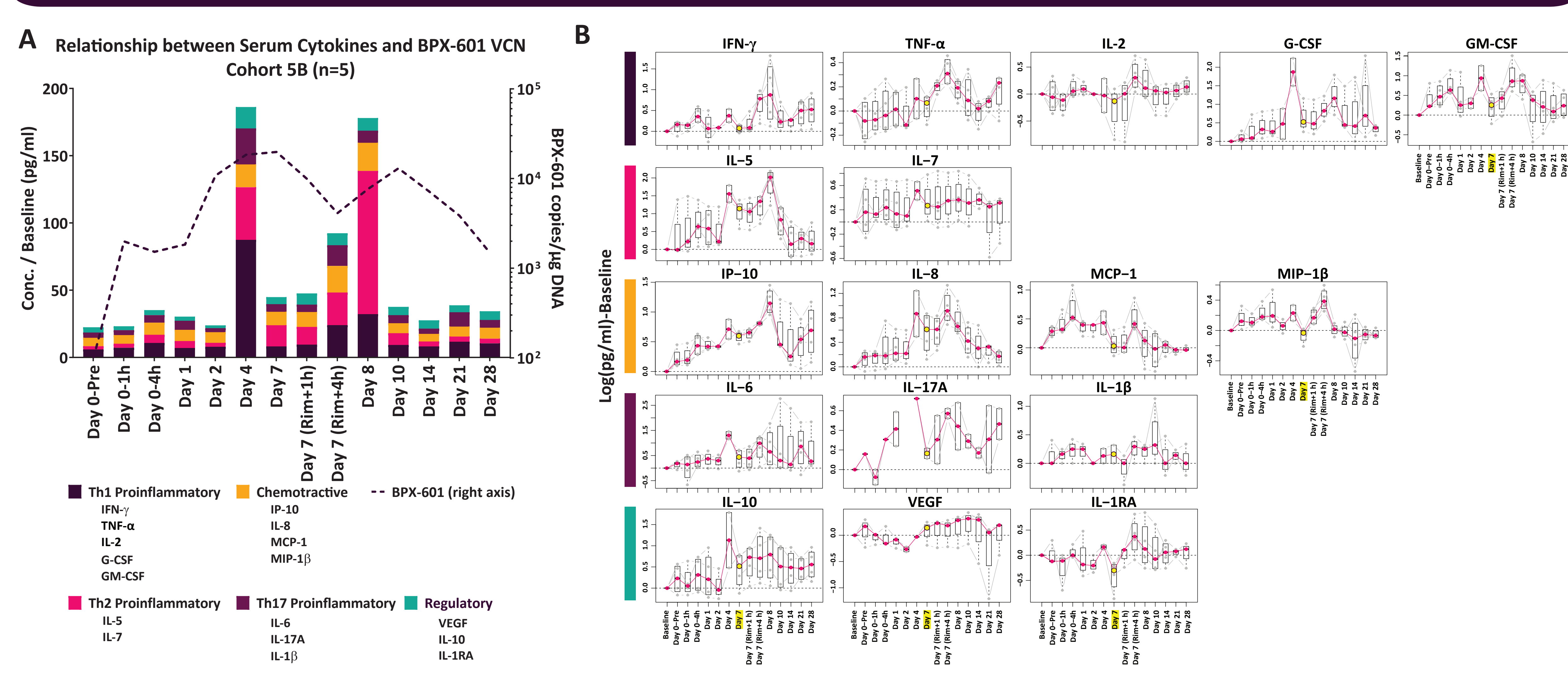


Figure 5. Serum cytokine levels in Cohort 5B patients (n=5) were evaluated at serial timepoints using a multiplex assay. (A) Analytes were grouped by immune function (Th1 proinflammatory, Th2 proinflammatory, and Regulatory) and the sum of mean cytokine levels at each timepoint was plotted in the stacked bars. The dotted line represents the mean VCN for Cohort 5B (n=5). Serum cytokine levels increased at Day 4 following BPX-601 infusion and within 24 hours of rimiducid infusion, with peak cytokine levels observed concurrently with increasing VCN. This data supports rimiducid-mediated T cell activation. (B) Serum concentration of individual analytes normalized to baseline was plotted, with mean levels from all Cohort 5B patients (n=5) shown in pink. Day 7 (pre-rimiducid) indicated by enlarged yellow data points. Data from individual patients are plotted (gray lines) along with the median and interquartile ranges (boxes).

## GoCAR-T CELLS INFILTRATED TUMOR METASTASES

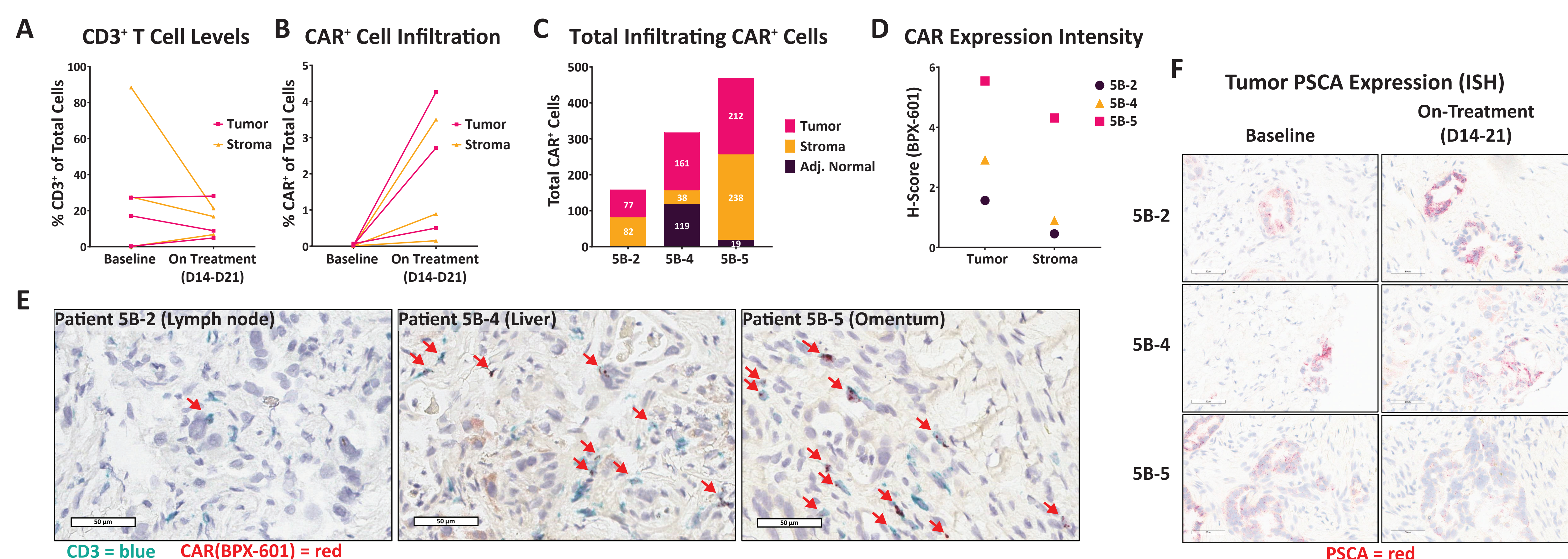


Figure 6. Biopsies taken at baseline and on-treatment (Day 14-21) from lymph node (5B-2), liver (5B-4) and omentum (5B-5) were stained for CD3 (IHC), BPX-601/CAR (RNAscope ISH), and PSCA (RNAscope ISH). (A) Percent CD3+ lymphocytes in tumor stayed relatively the same between baseline and on-treatment. (B) Infiltration of CAR+ cells into tumor tissue was observed in all on-treatment specimens (n=3). (C) CAR+ cells were located both in the tumor and the stroma in all patients (n=3). (D) The highest intensity of CAR staining was observed in patient 5B-5, who also had the highest number of infiltrating CAR+ cells. (E) CAR+ cells were observed proximal to tumor, representative images; CD3 (blue) and CAR (red). (F) PSCA expression was observed in baseline and on-treatment specimens, representative images showing PSCA (red).

## DIFFERENTIAL GENE EXPRESSION IN TUMOR MICROENVIRONMENT

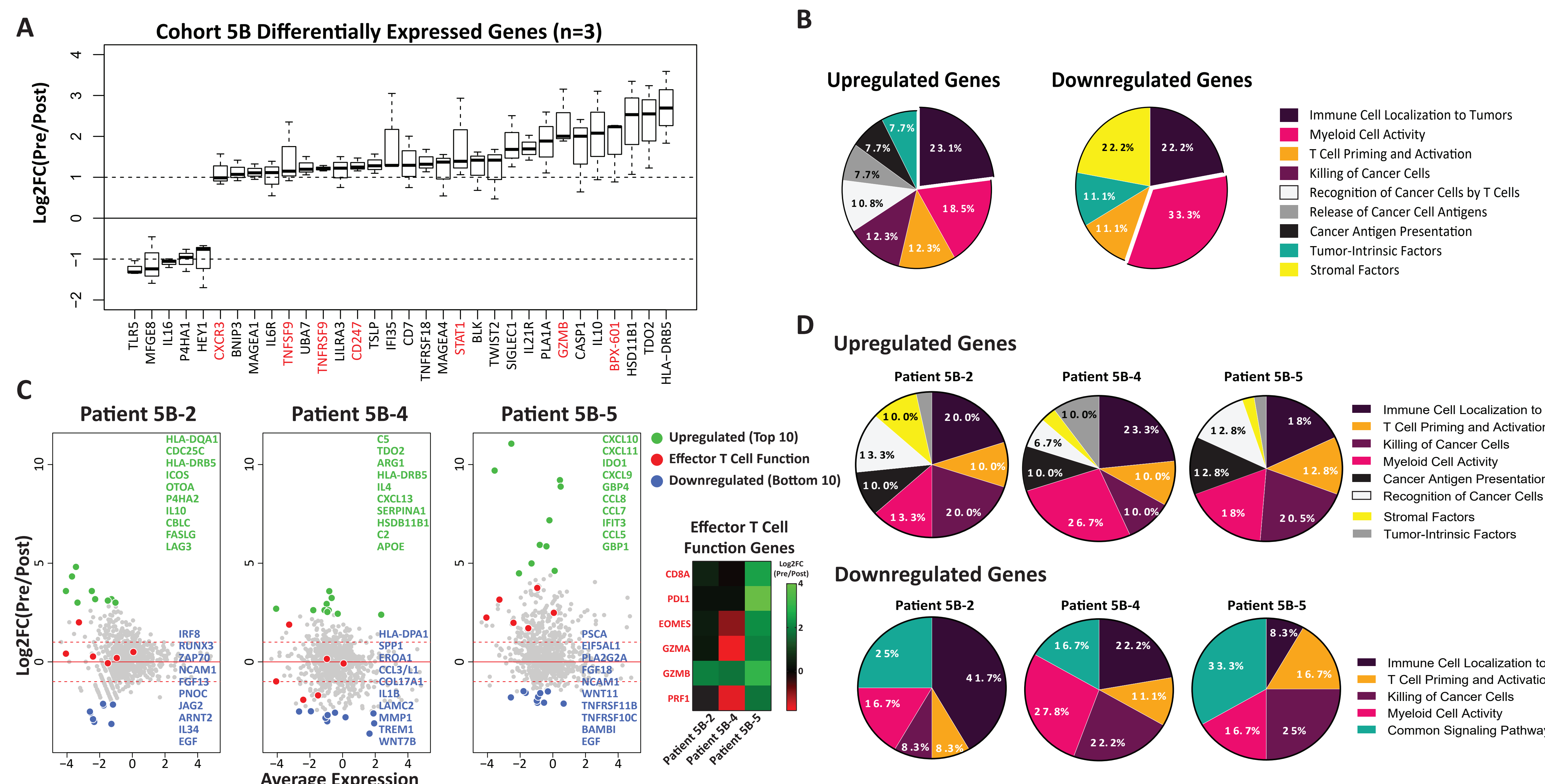


Figure 7. Differential gene expression in response to treatment with BPX-601 and rimiducid were evaluated in available biopsies from cohort 5B patients (n=3) using the Nanostring PanCancer IO 360 panel. (A) The log fold change in gene expression between the baseline and on-treatment samples was evaluated and consistent changes were observed in all patients (n=3, p < 10%). Genes highlighted in red are potentially associated with CAR-T activity. (B) The relative contribution from genes in defined functional categories to differential gene expression observed in all 3 patients. Upregulated genes were most associated with immune cell localization to tumors, while downregulated genes were most associated with myeloid cell activity. (C) Individual gene expression profiles for each patient evaluated (n=3). Upregulation of interferon-responsive genes was observed in patient 5B-5, together with genes associated with effector T cell function and downregulation of PSCA which is indicative of a productive, effector T cell response. Patient 5B-5 also had the highest levels of tumor infiltrating BPX-601 cells (Figure 6B-C). (D) Functional annotation of individual patient gene expression profiles revealed a diverse pattern of functional attributes.

## CONCLUSIONS

- BPX-601 GoCAR-T cells exhibited enhanced survival and persistence up to 9 months.
- Activation of BPX-601 GoCAR-T cells mediated upregulation of immunomodulatory cytokines in patients.
- BPX-601 GoCAR-T cells infiltrated metastatic pancreatic tumors.
- Changes in tumor microenvironment gene expression consistent with a productive CAR-T cell immune response were observed in patients treated with BPX-601 GoCAR-T cells activated with rimiducid.
- Patients are currently being enrolled in BP-012 study Cohort 5C to assess the safety and efficacy of repeated BPX-601 activation with weekly rimiducid administration.

The authors would like to acknowledge all patients, their families, and caregivers for participating in this clinical trial, along with the investigators and their staff.



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