

Synergy Between BXCL701, a DPP Inhibitor, and Immune Checkpoint Inhibitors Discovered Using AI and Big Data Analytics

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INTRODUCTION

EvolverAI artificial intelligence (AI) enabled platform, designed to optimize therapeutic value of diseases, disease biology, therapeutic modalities and drugs. Multiple therapeutic compounds have been identified using EvolverAI platform with potential to act synergistically in combination with immune-checkpoint inhibitors (ICI) by remodelling the tumor micro-environment and transforming cold, non-inflamed tumors into hot immune-sensitive tumors.

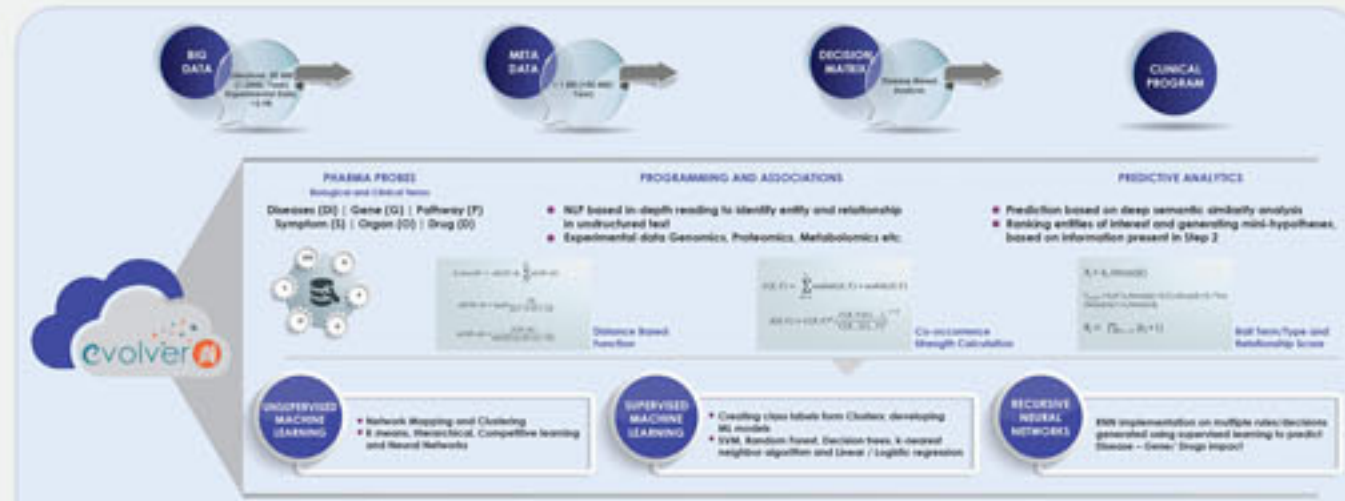


Figure 1: EvolverAI - BioXcel's in-house artificial intelligence enabled platform

BXCL701 previously known as Talabostat/PT-100, transforms the immune-deserted tumor-milieu conducive to immune-inhabitation by targeting FAP, DPP8 and DPP9. More than 700 humans have received BXCL701 in series of clinical trials giving a comprehensive clinical overview of safety, PK, target inhibition and downstream PD effect on cytokine increase and immune-cell modulation. Further, existing data indicates that inhibition of these targets of BXCL701 can affect NEPC at multiple levels, indicating its use as a novel therapeutic in neuroendocrine prostate cancer (NEPC). NEPC is an aggressive tumor that appears in about 20-30% of patients progressing after treatment with androgen inhibitors Zytiga and Xtandi (ADT) with limited therapy options.

Existing data shows that inhibition of DPP8/9 and Fibroblast Activator Protein (FAP) can affect NEPC at multiple levels:

- NPY, a neuroendocrine peptide hormone upregulated in NEPC, is a substrate of DPP8-9. NEPC-like cells use NPY to grow and survive and blocking the processing of NPY results in antitumor activity
- FAP+ cancer associate fibroblast (CAF) are present in the tumor micro-environment and are activated by ADT. Depleting FAP+CAF delay or prevent CRPC development.
- FAP+CAF together with MDSC block CD8+ T-cell infiltration into the prostate tumor microenvironment, one of the major reason for the poor response to immune-checkpoint inhibitors (ICI) monotherapy in CRPC. BXCL701 not only can deplete FAP+CAF but induces a granulocytic differentiation resulting in less immune suppressive MDSC and neutrophil infiltration, which synergistically increase ICI antitumor activity
- Finally, BXCL701 triggers the macrophage cell death "pyroptosis" resulting in proinflammatory stimulation of the innate immunity leading to defining DPP8/9 as "novel immune-checkpoints".
- BXCL701 stimulates the priming, migration and cytotoxicity of T-cells and the formation of memory T-cells

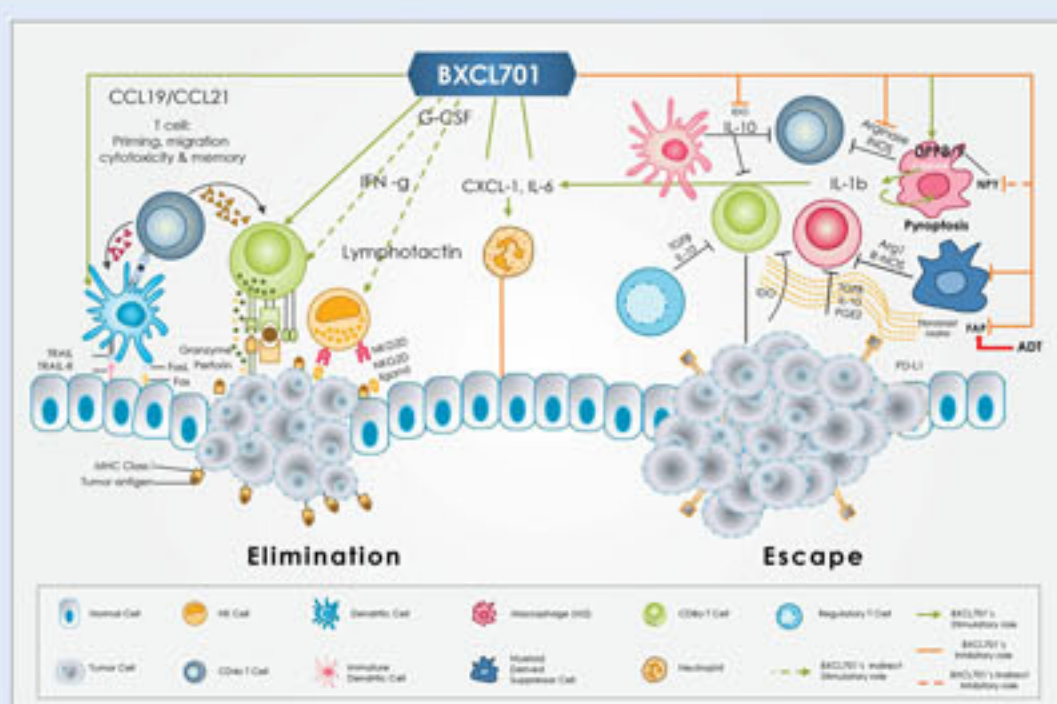


Figure 2: BXCL701 influencing cancer-immune cycle and its dual role in immuno-oncology

OBJECTIVES

- To identify in-silico hypotheses for synergistic combinations in ICI therapy based on public domain literature and experimental dataset using EvolverAI
- To validate experimentally the EvolverAI-prioritized compounds by testing their antitumor activity in combination with anti-PD1 in syngeneic mouse models and define the biochemical and cellular mechanism for synergy
- To use immune-genomics approaches to prioritize tumors for BXCL701 and anti-PD1 combination therapy

KEY FINDINGS ...

A. EvolverAI IO test case: BXCL701 as a potential combination agent for ICI therapy

EvolverAI leveraged techniques like natural language processing, co-occurrence scores and relationships mapping to unravel novel connections between compounds, genes, immune modulation and cancer types and generate combination hypothesis[ref.1, A1]. These hypotheses were prioritized based on a proprietary matrix scoring the biological relevance of the mechanism and the level of clinical validation, safety concerns and opportunity for biomarker use [A2]. Using SysBiotics, a protein interaction map of BXCL701 targets with PDL1 identified 5 transcriptional modulators with link to prostate cancer -GATA2 (prostate cancer aggressiveness [ref.2]); C/EBP (proliferative inflammatory atrophy of the prostate [ref.3]); STAT3 (target and biomarker in advanced prostate cancer [ref.4]); EGR1 (gene expression modulation in CRPC [ref.5]) and CTCF (mutations associated with prostate cancer [ref.6]) [A3].

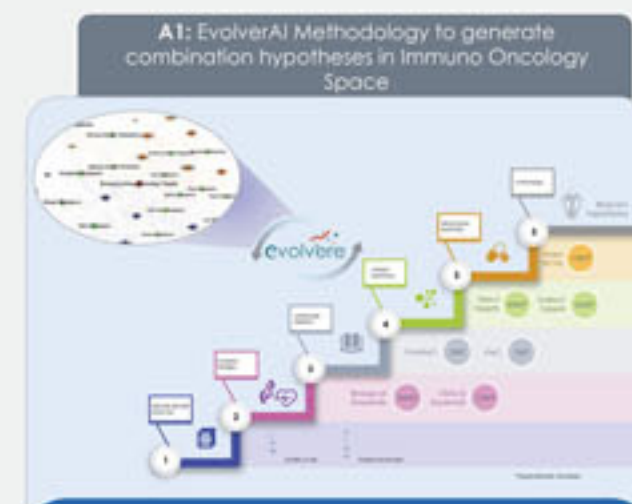


Figure 3: EvolverAI methodology to identify potential repurposing opportunities in immuno-oncology space



Figure 4: Hypotheses prioritization based on mechanism, liability, target-validation, biomarkers potential, and commercial attractiveness.

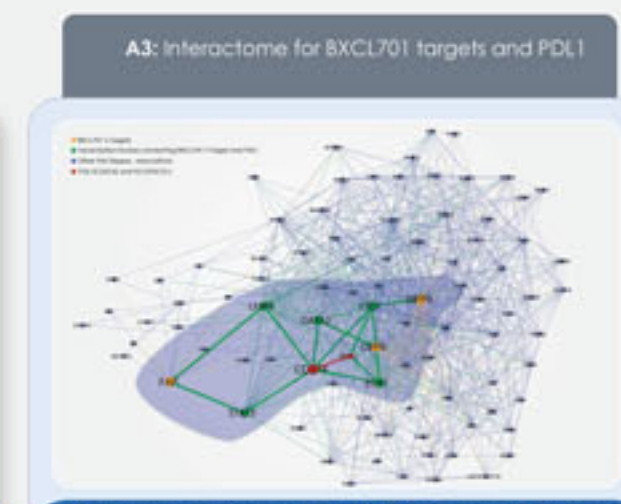


Figure 5: Interaction Network of BXCL701 targets (as orange nodes) with PDL1/CD274 (as green nodes) through 5 transcription factors (as green nodes) using Cytoscape v3.3.0 by Biogenet v.3.0 plugin.

B. Experimental Validation- for BXCL701 with anti-PD1 in M38 syngeneic mouse model

MC38 mouse colon cancer cell line (1.0x10⁶ MC-38 cells/ 0.1ml Hanks Balanced Salt Solution) were implanted in the subcutaneous space of the flank of mice of female C57BL/6 mice. In the first experiment [B1a] tumors grow slower and all treatment groups had statistically higher anti-tumor activity compared to controls and BXCL701 and anti-PD1 antibody combination had higher efficacy than the single agent treatments. In the second experiment [B1b], tumor grew much faster. None of the single agent treatment had statistically higher anti-tumor activity compared to controls but BXCL701 and anti-PD1 antibody combination had significant antitumor activity and 10 µg BID and 20 µg QD showed comparable efficacy. Upregulation of pro-inflammatory cytokines (IL-2, IL-6, IL-12p40), immunostimulatory chemokines (GM-CSF, G-CSF) and cytokines predicting memory T-cell response (IL-15 and IL-7) were observed post-treatment [B2]. Immunophenotyping revealed that the combination of BXCL701 with anti-PD1 increased the percentage of the cytotoxic NK cells and macrophages in the tumor and blood respectively while decreased in the immunosuppressive T regulatory cells [B3].

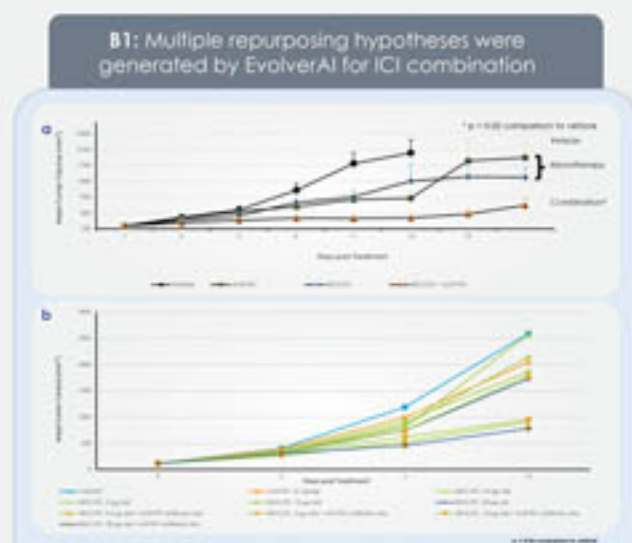


Figure 6: Comparing treatment effect of BXCL701 with anti-PD1 (combination), BXCL701 (single agent), anti-PD1 (control), vehicle (control) on mean tumor volume in syngeneic mouse model - MC38 in C57/BL6 mice

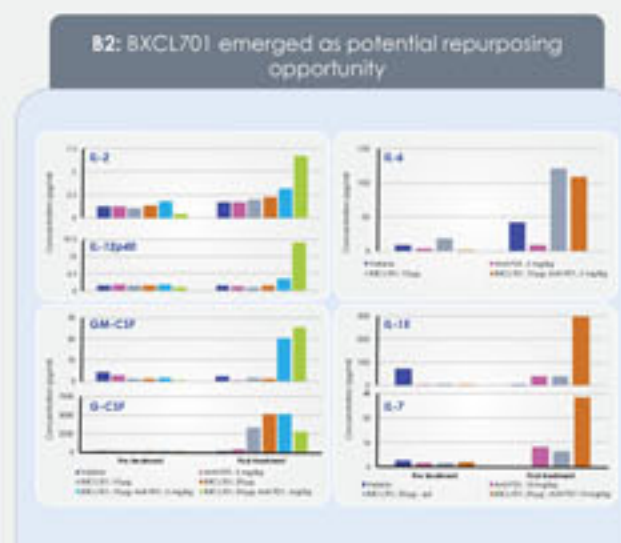


Figure 7: Comparing immunomodulatory effects by Luminex assay for (a) pro-inflammatory cytokines (b) chemokines and (c) associated memory T-cells by dose administration of QD, BID as single agent and in combination with anti-PD1

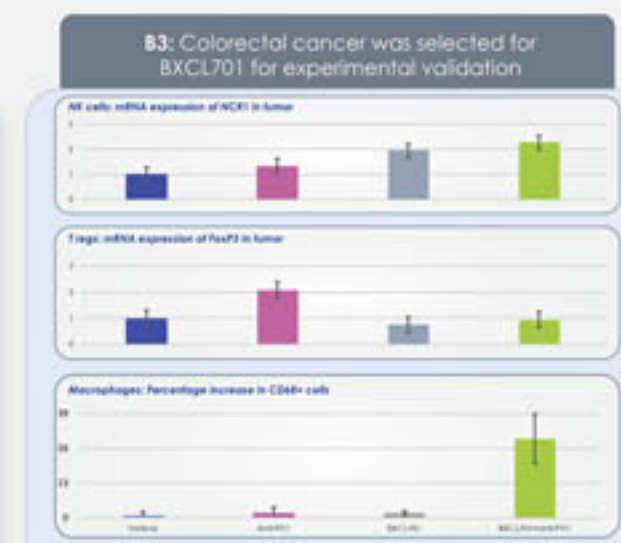


Figure 8: Comparing percentage of NK cells, macrophages and T-regulatory cells in tumor and blood samples by immunophenotyping for anti-PD1, BXCL701 and BXCL701 + anti-PD1 combination

C. Analysis of genomic alterations in FAP, DPP8 and DPP9 across cancer genomic profiles

Genomic alterations in FAP, DPP8 and DPP9 were analyzed across 33172 patients from 150 different cancer studies included dataset from TCGA at cBioPortal identified castration-resistant prostate cancer patients pretreated with Abiraterone and Enzalutamide (ref.7-9) as an outlier. High level amplification of DPP9 (14%) and overexpression of DPP8 in 50% of CRPC suggests that this patient population could be uniquely sensitive to this monotherapy treatment. Neuropeptide Y (NPY) is a substrate of DPP8/9, it is highly upregulated in prostate cancer (see figure 9b). NPY selectively stimulates the growth of a NEPC-like cancer cell line and DPP8/9 inhibition decreases tumor cell survival because of a decreased cleavage of NPY. In addition the almost complete overlap of the overexpression of DPP8 and PDL1 suggests could be sensitive to combination treatment.

KEY FINDINGS ...

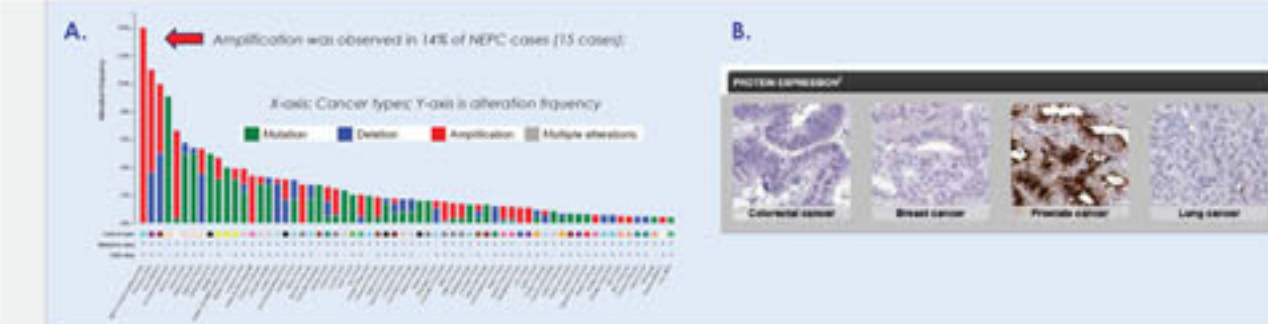


Figure 9: A. Summary of genomic alterations - mutations, deletions, amplifications and multiple alteration - across cancers for BXCL701 targets (Accessed from cBioPortal version 1.3.2; on 8 November, 2016) B. Immunohistochemistry image of Neuropeptide Y (NPY), a substrate of DPP8/9, shown to be upregulated in prostate cancer



Figure 10: OncoPrint representation of Prostate cancer dataset of Beltran et al, 2016 highlights co-amplification and over expression of BXCL701 targets DPP8 and DPP9 with PDL1 (CD274). Each row represents a gene and columns represent each sample. (Accessed from cBioPortal Version 1.4.3 as on 9th March, 2017)

CONCLUSIONS

Demonstration of the capability of EvolverAI, an artificial intelligence driven big data analytics platform to generate in-silico hypotheses for synergistic combinations in ICI therapy

Validation of the synergistic impact of BXCL701 and anti-PD1 combination in a syngeneic mouse model for colon adenocarcinoma

Identification of exciting opportunity for BXCL701, alone and in combination with anti-PD1 therapy in prostate cancer patients based on landscape analysis of target expression profiling

FUTURE PERSPECTIVE

Given these strong mechanistic and preclinical data, an open label clinical trial is being planned to test the antitumor activity of BXCL701 either as monotherapy or in combination with Keytruda in patients with NEPC. This POC trial, using Simon-2 stage design and leveraging existing clinical PK/PD and safety data to define upfront a dose and regimen will clarify whether BXCL701 can make a difference for NEPC patients in a fast and efficient way.

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