Discovery of best-in-class dual-acting $A_{2A}R/A_{2B}R$ antagonists that are efficacious in high adenosine tumor microenvironment

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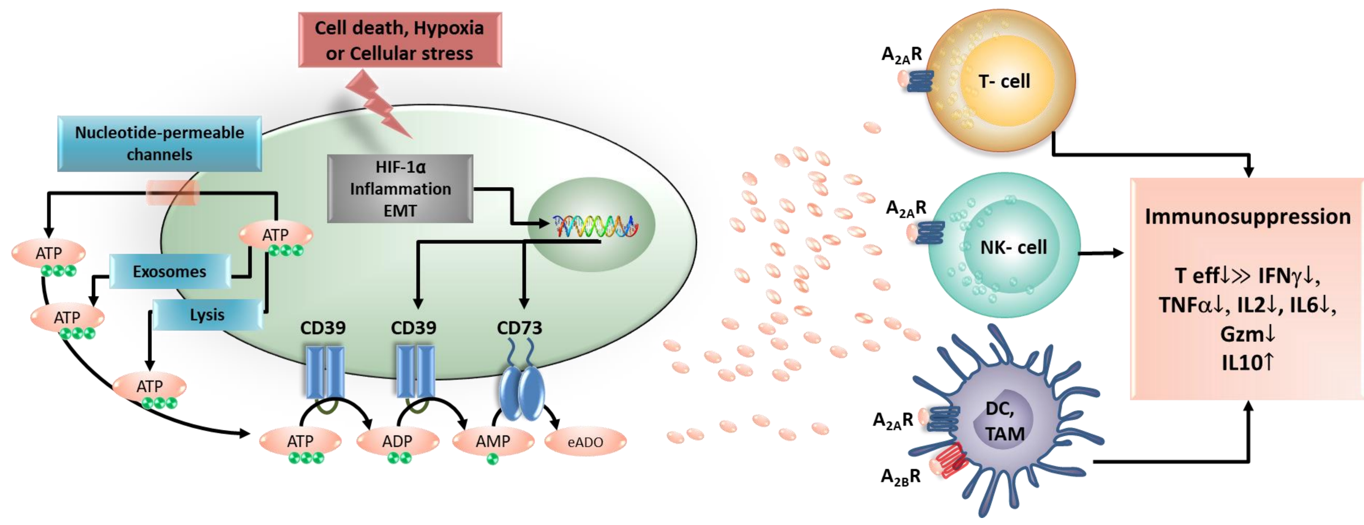
Nainesh Katagihallimath[1], Radha Nandishaiah[1], Sreevalli Sharma[1], Nishanth Belugali Nataraj[1], Purnendu Bhowmik[1], Suryanarayanan Venkatesan[1], Ranga Rao[1], Nagakumar Bharatham[1], Mehak Gupta[1], Debosmita Kundu[1], Harish K Kotakonda[1], Rajeshkumar RK[1], Sambasiva Reddy[1], Biplab Tewary[1], Seema Muley[2], Akshay Kudpaje[2], Aparna Gangoli[2], Adithya V. Naragund[2], Poovamma C Uthappa[2], Upasana Mitra[3], Subrat Mohapatra[3], Shweta Kapoor[3], Leela Maitreyi[1], V. Balasubramanian[1], Santanu Datta[1], Shashikala Vinayakamurthy[1], Biswanath Majumder[1], Shahul Hameed P[1]

[1]Bugworks Research India Pvt. Ltd., Bengaluru, India, [2]Cytecare Cancer Hospitals, Bangalore, India, [3]Cellworks Research India Pvt. Ltd., Bangalore, India

email: nainesh@bugworksresearch.com

Introduction

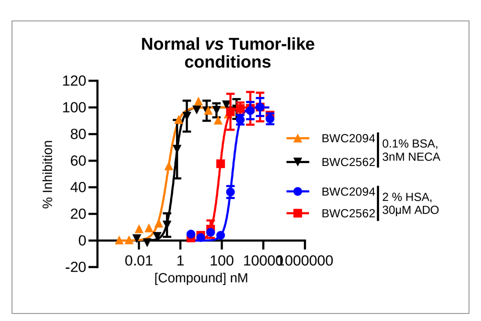
Adenosine accumulates in the tumor microenvironment due to abnormal tumor metabolism and hypoxia. It binds to A_{2A} and A_{2B} receptors on immune cells and impairs their activation and cytotoxic ability, helping cancers to evade the immune system and resist cancer therapies. Adenosine receptor antagonists that act in **pathological concentrations of adenosine** can rescue T cell and innate immune suppression to improve the efficacy of existing treatments.

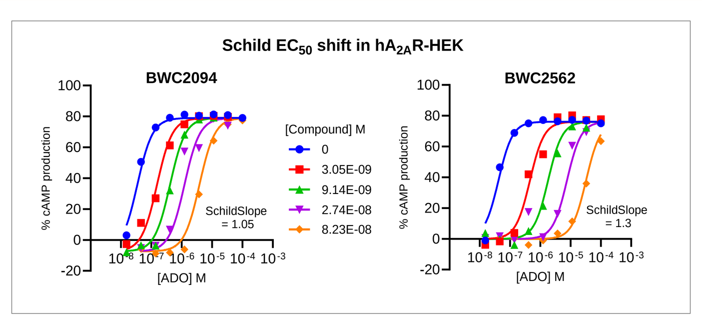


Summary

BWC2094 & BWC2562 are potent A_{2A}/A_{2B} dual antagonists that are efficacious in a high adenosine environment. They selectively bind A_{2A} at picomolar concentrations and have >50-fold selectivity over A_1 and A_3 receptors. They both reduced phosphorylation of CREB and restored the production of cytokines, such as IL2, TNFα and IFNγ in adenosine-suppressed human PBMCs. These compounds exhibited synergy with several approved drugs in co-culture assays of tumor cells and PBMCs. Both, BWC2094 and BWC2562 demonstrated good oral bioavailability in rodents (>25 %F) and dogs (>50 %F) and were well tolerated for 14 days in rat acute toxicity studies. At a dose of 3mg/kg, they reduced the tumor volume as monotherapy and enhanced the anti-tumor effects of anti-PD1/PDL1 and CTLA4 in CT26, MC38, and MCA205 murine tumor models. In a patient-derived ex vivo tumor explant platform, they reduced tumor density and Ki67 expression, and increased apoptosis/necrosis.

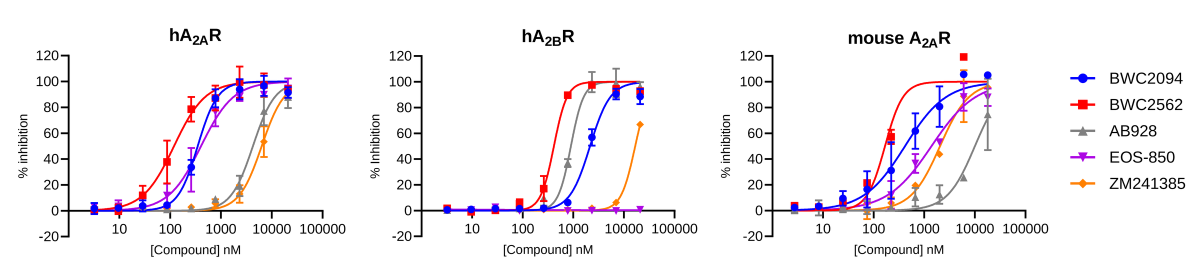
Potent under high adenosine tumor microenvironment





$hA_{2A}R$-HEK cells were stimulated under normal (low adenosine/5nM NECA) and tumor-like (30μM adenosine, 2% HSA) conditions for 30 minutes. cAMP was measured to calculate antagonist potencies. Adenosine dose response was performed with increasing concentrations of BWC2094 and BWC2562 for Schild EC_{50} shift analysis.

Dual antagonism against A_{2A} and A_{2B} receptors



Potency, IC_{50} (nM)	BWC2094	BWC2562	AB928	EOS-850	ZM241385
Human $A_{2A}R$	320	70	2381	272	6262
Human $A_{2B}R$	2143	414	897	> 20000	16735
mouse $A_{2A}R$	715	225	15300	1635	7041

$hA_{2A}R$, $hA_{2B}R$ HEK and $mA_{2A}R$ CHO cells were stimulated with 30μM adenosine in the presence of 2% (w/v) HSA & 30μM EHNA. Inhibition of cAMP production by antagonists was measured using TR-FRET assay. Plots: mean of 3 independent experiments.

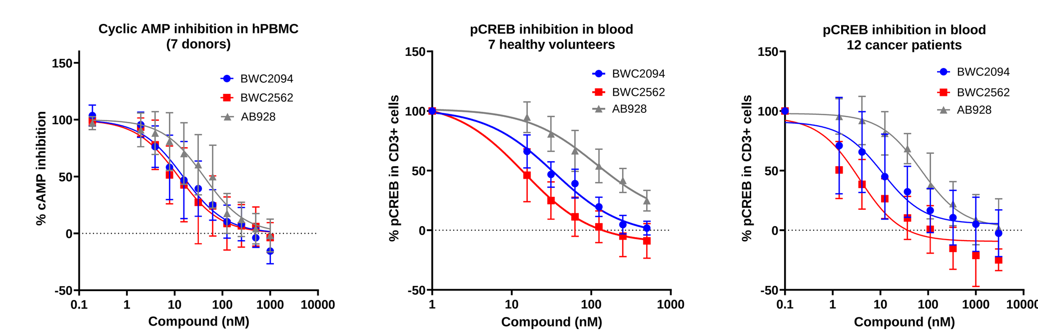
Selectivity against A_1 and A_3 receptors (physiological conditions)

Potency, IC_{50} (nM)	BWC2094	BWC2562	AB928	EOS-850
$hA_{2A}R$ (NECA)	0.25	0.3	2	0.31
$hA_{2B}R$ (NECA)	1.4	3	1.5	> 10000
hA_1R (CPA)	18 (72-fold)	240 (800-fold)	102 (51-fold)	581 (558-fold)
hA_3R (IB-MECA)	102 (408-fold)	30 (100-fold)	529 (265-fold)	174 (1874-fold)

A_1R, A_3R Hunter cells (10μM Forskolin) and $A_{2A}R$, $A_{2B}R$ HEK cells with $[EC_{80}]$ of respective agonists were used to monitor the receptor activity via cAMP measurements. Fold selectivity in parentheses are ratios with respect to $A_{2A}R$ potencies. NECA = 5′-(N-Ethylcarboxamido)adenosine, CPA = Cyclopentyladenosine, N6-(3-Iodobenzyl)adenosine-5′-N-methyluronamide.

- BWC2094 & BWC2562 are potent dual antagonists of $A_{2A}R$ and $A_{2B}R$ at tumor-like high adenosine and in high plasma protein environment
- Schild EC_{50} shift analysis demonstrates competitive antagonism with $hA_{2A}R$
- Selective against the G_i adenosine receptors, namely A_1R and A_3R
- BWC2094 & BWC2562 outperform competitor adenosine antagonists in clinical development

Potent inhibition of pCREB in PBMCs (clinical PD biomarker)

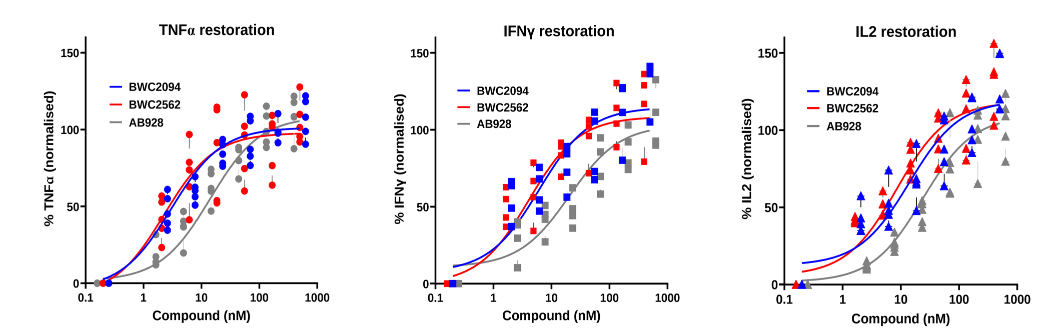


Activated PBMCs were treated with test compounds and 5μM NECA for 30min. cAMP was measured by a TR-FRET assay. Whole blood from 7 healthy donors and 12 cancer patients was treated with test compounds along with 5μM NECA for 60 min at 37°C, phosphorylated CREB was analyzed by flow cytometry in CD3 cells.

Potency, EC_{50} (nM)	BWC2094	BWC2562	AB928
Healthy donor, cAMP	15	12	41
Healthy donor, pCREB	34	14	115
Cancer patient , pCREB	11	3.7	73

- BWC2094 & BWC2562 efficiently modulate cAMP levels in immune cells
- They demonstrate improved target engagement ex vivo in cancer patient blood

Cytokine restoration under immunosuppressive conditions

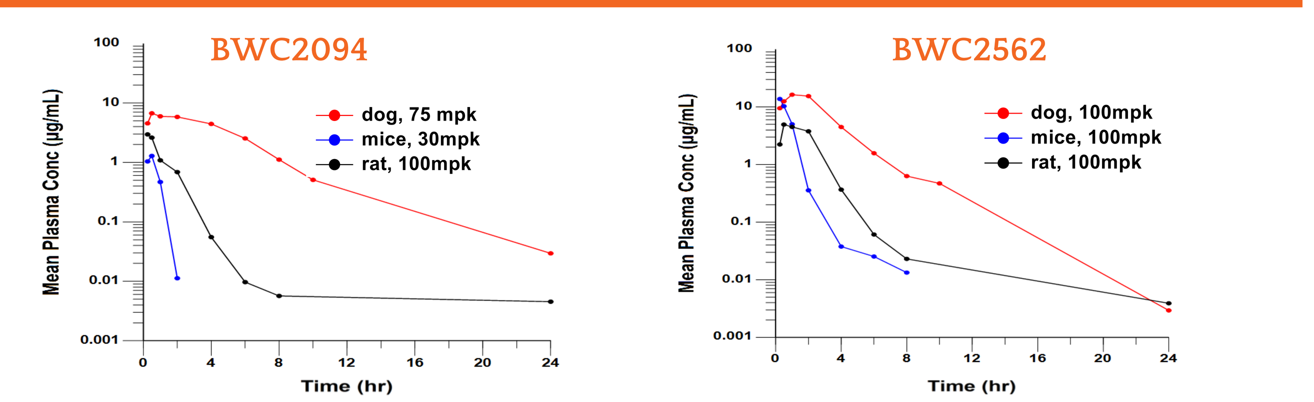


Activated PBMCs were treated with test compounds and NECA for 48hrs. Cytokines were quantified in cell-free supernatant by AlphaLISA. Mean EC_{50} values from 5 healthy donors is presented in the plot and table below.

Potency, EC_{50} (nM)	BWC2094	BWC2562	AB928
TNFα	3	2.3	14
IFNγ	6	4	20.4
IL2	14	8.6	23.8

- BWC2094 & BWC2562 restore cytokine production in T-cells through adenosine receptor antagonism

Favorable pharmacokinetic characteristics



Species	Dose mg/kg	C_{max} μg/mL	T_{max}* hr	AUC_{0-t} h*μg/mL	AUC_{0-inf} h*μg/mL	%F**
BWC2094 dosed via oral route						
Mice /CD1	30	0.24 ± 0.14	0.25 (0.25-0.25)	0.14 ± 0.78	0.15 ± 0.08	<5
Rat /SD	100	3.33 ± 1.15	0.25 (0.25-0.5)	3.35 ± 1.61	3.35 ± 1.61	14
Dog /Beagle	75	7.45 ± 2.01	1.0 (0.5-2.0)	36.89 ± 11.58	37.04 ± 11.67	>90%
BWC2562 dosed via oral route						
Mice/CD1	100	13.79 ± 3.98	0.25 (0.25-0.25)	10.50 ± 1.12	10.52 ± 1.12	7.7
Rat /SD	100	4.96 ± 0.59	0.5 (0.5-0.5)	11.18 ± 0.32	11.21 ± 0.31	22 ± 4.4
Dog/ Beagle	100	17.93 ± 4.23	1.0 (1.0-2.0)	59.74 ± 18.25	59.74 ± 18.25	47 ± 9.0

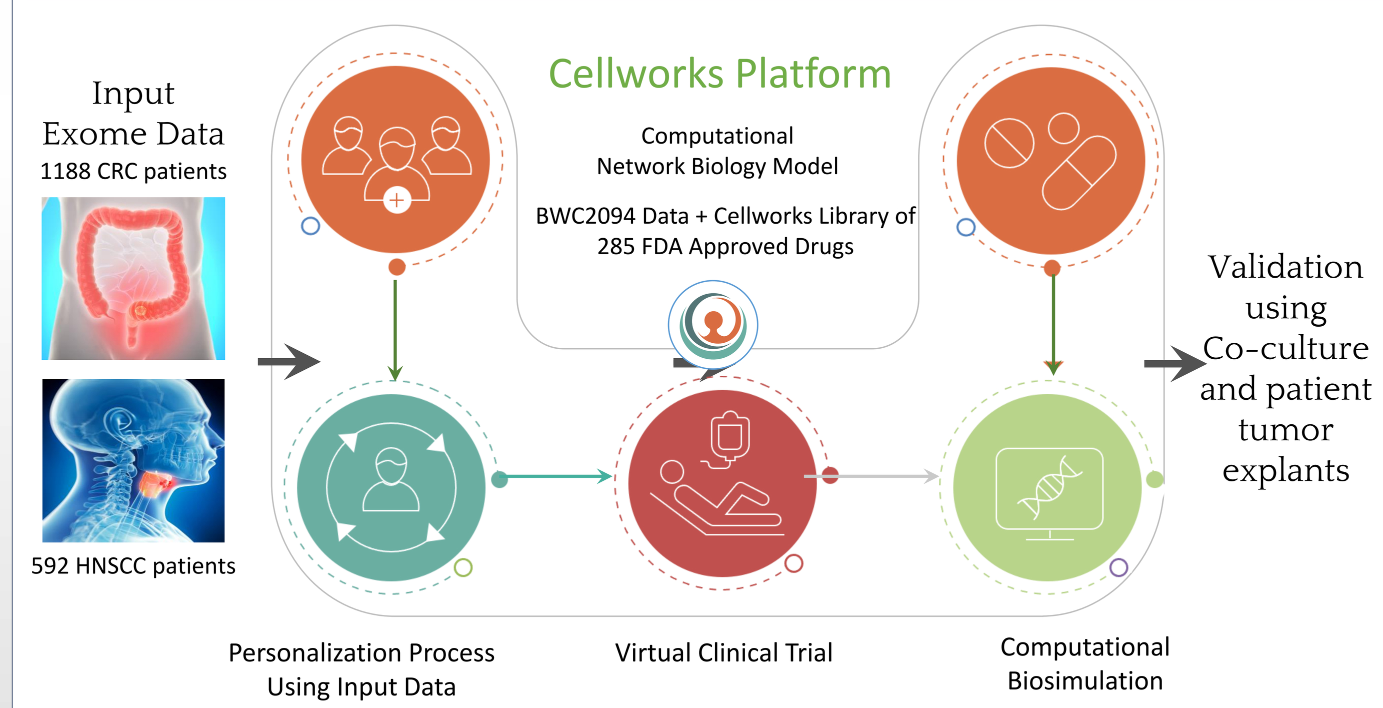
BWC2094

- BCS Class-II (highly permeable with low-moderate pH-dependent solubility)
- 10% free fraction across species
- Total systemic clearance is equal to that of hepatic clearance and the compound is highly distributed
- Oxidative metabolism by CYP3A4 , CYP1A2 and CYP2C19
- No food effect observed in dogs
- Well tolerated in rat and dog till 300mpk when dosed once daily for 14 days
- The predicted clinical dose based on the PK/PD model is 75mg BID

BWC2562

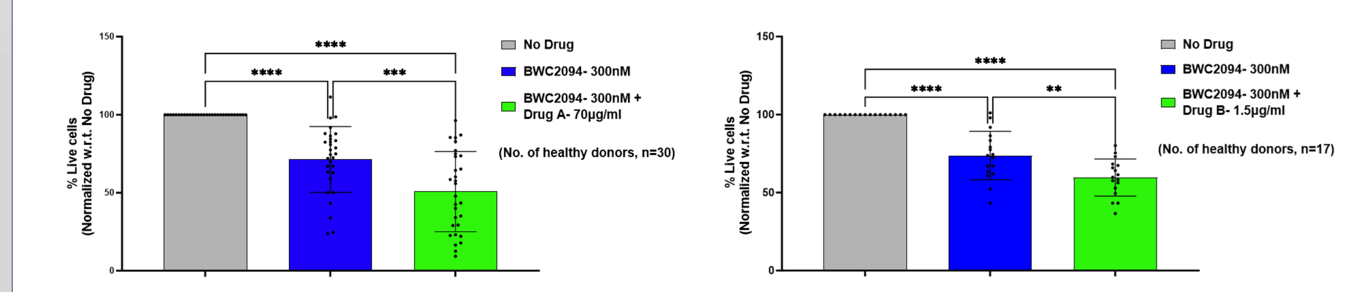
- BCS Class-II (highly permeable with low-moderate solubility)
- 2-14% free fraction across species
- Metabolically stable across species (based on in vitro Hep clearance) observed in the PK across species
- Good oral bioavailability (%F) in rats (22%) and dogs (47%)
- Well tolerated in rat and dog for 14 days once daily till 300 mpk
- The predicted clinical dose based on the PK/PD model is 50mg BID

AI/ML driven virtual trials to identify combinations, and patient selection



Validation of AI/ML virtual trial in a co-culture assay

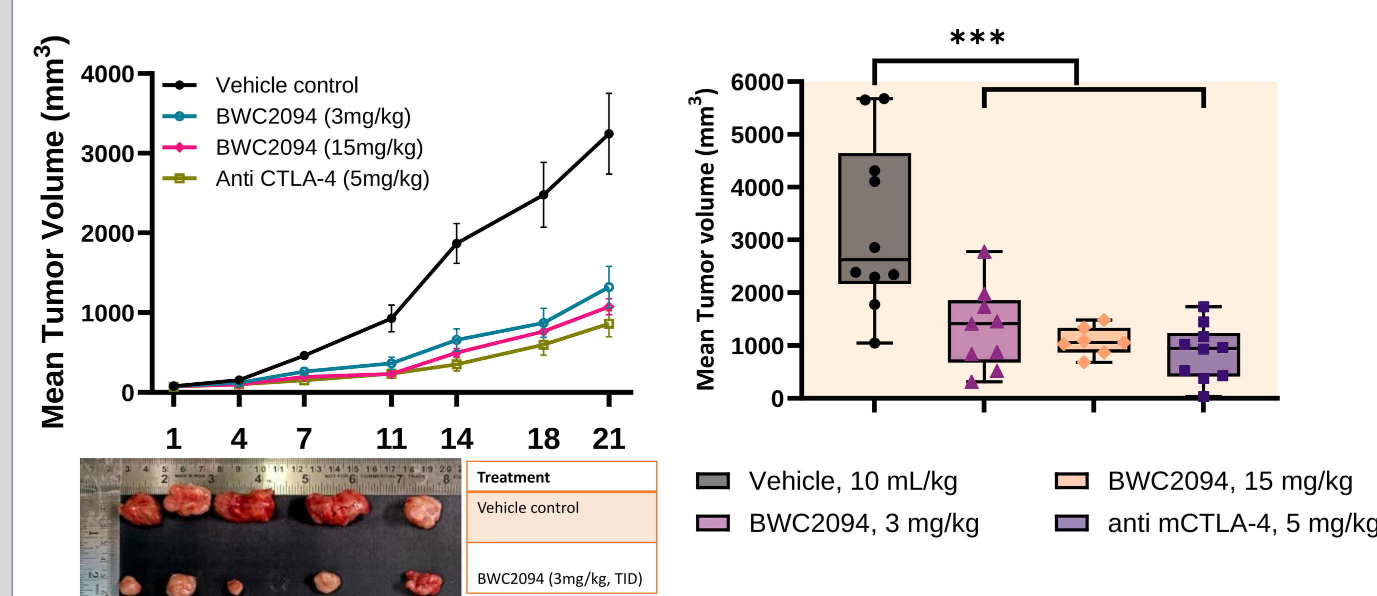
Synergy with approved drugs in co-culture



- In co-cultures of tumor cells with activated PBMCs, BWC2094 showed significant synergy by enhanced tumor killing, both as stand alone & in combination with drugs A & B

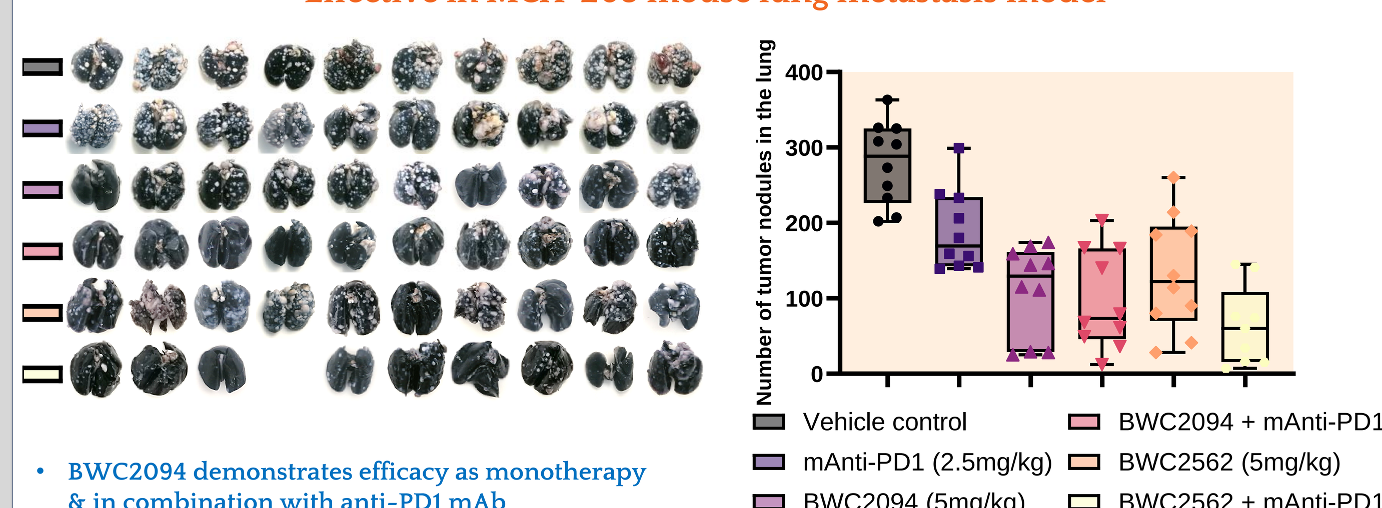
Significant tumor growth inhibition in mouse models

CT-26: colon cancer mouse model recapitulates a high adenosine environment



- Robust efficacy demonstrated for BWC2094 in CT26 syngeneic mouse model that has adenosine concentrations of about 100μM in the tumor microenvironment
- Observed efficacy is comparable to anti-CTLA-4 treatment

Effective in MCA-205 mouse lung metastasis model

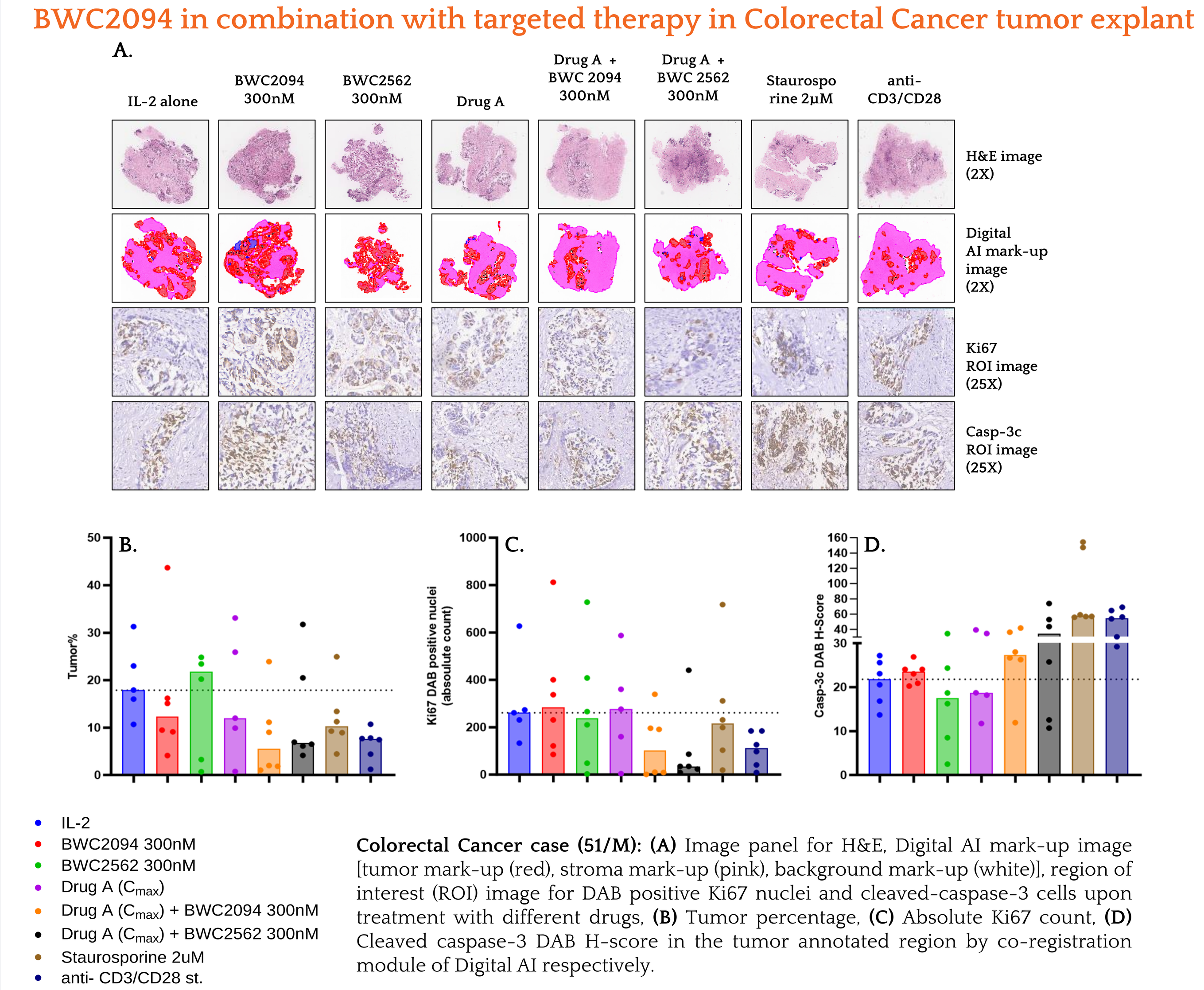


- BWC2094 demonstrates efficacy as monotherapy & in combination with anti-PD1 mAb

Confirmation of virtual trial outputs

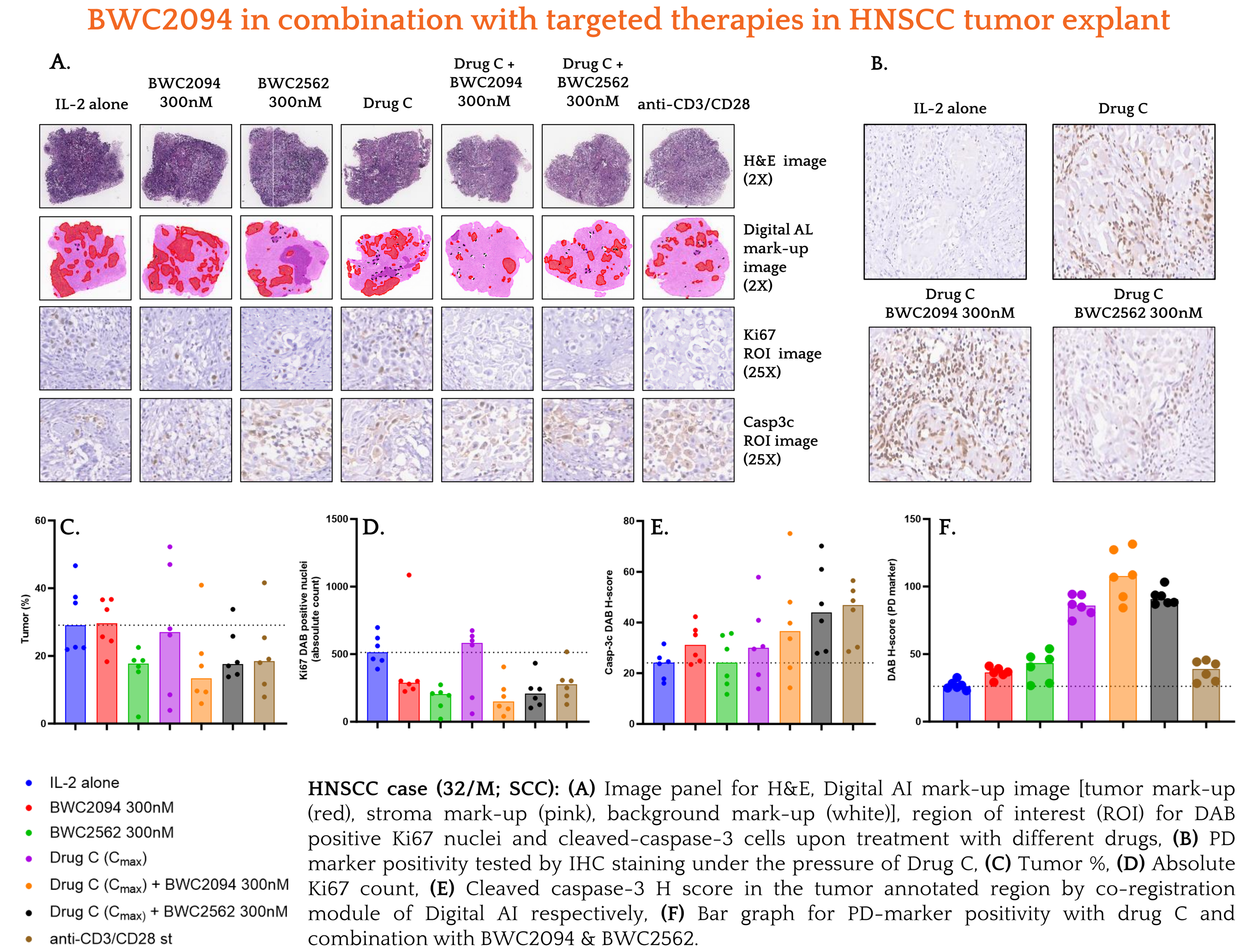
An in-house platform leveraged to decipher the impact of combining BWC2094 & BWC2562 with targeted drugs in patient derived ex-vivo system (CRC and HNSCC patients) using AI driven digital pathology and IHC profiling.

BWC2094 in combination with targeted therapy in Colorectal Cancer tumor explant



Colorectal Cancer case (51/M): (A) Image panel for H&E, Digital AI mark-up image [tumor mark-up (red), stroma mark-up (pink), background mark-up (white)], region of interest (ROI) image for DAB positive Ki67 nuclei and cleaved-caspase-3 cells upon treatment with different drugs, **(B)** Tumor percentage, **(C)** Absolute Ki67 count, **(D)** Cleaved caspase-3 DAB H-score in the tumor annotated region by co-registration module of Digital AI respectively.

BWC2094 in combination with targeted therapies in HNSCC tumor explant



HNSCC case (32/M; SCC): (A) Image panel for H&E, Digital AI mark-up image [tumor mark-up (red), stroma mark-up (pink), background mark-up (white)], region of interest (ROI) for DAB positive Ki67 nuclei and cleaved-caspase-3 cells upon treatment with different drugs, **(B)** PD marker positivity tested by IHC staining under the pressure of Drug C, **(C)** Tumor %, **(D)** Absolute Ki67 count, **(E)** Cleaved caspase-3 H score in the tumor annotated region by co-registration module of Digital AI respectively, **(F)** Bar graph for PD-marker positivity with drug C and combination with BWC2094 & BWC2562.

- BWC2094 & BWC2562 in combination with Drug A & Drug C have shown reduction in tumor %, decrease in Ki67 and corresponding increase in cleaved Caspase-3

Conclusions

- BWC2094 & BWC2562 are dual-acting antagonists targeting A_{2A} and A_{2B} receptors, even at elevated adenosine levels that are seen in the TME.
- In the immune cells, they modulate the secondary messenger cAMP, engage the clinical biomarker pCREB and restore production of pro-inflammatory cytokines.
- Efficacious in reducing tumor volume in multiple murine models.
- We identified novel combination drug partners for BWC2094 & BWC2562 using AI/ML that were validated and confirmed through co-culture and human tumor explant assays.
- Demonstrated adequate safety in 14-day rat and dog studies.
- Bugworks compounds have the potential to significantly improve the clinical outcomes in solid tumors such as in CRC, NSCLC, TNBC, HNSCC, Ovarian cancer and RCC.

Disclosure: Nainesh Katagihallimath is a full-time employee of Bugworks Research India Pvt. Ltd. and holds employee stock options. email: nainesh@bugworksresearch.com