Biochemical and Cellular Evaluation of HUNK Inhibition by an FDA Approved Drug as Potential Therapeutic Strategy for HER2+ Breast Cancer

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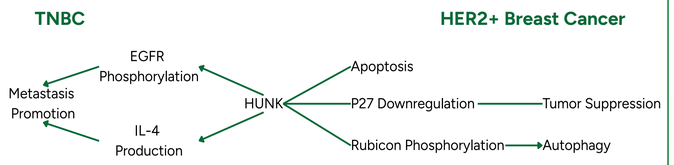
Introduction

Hormonally Up-regulated Neu-associated Kinase (HUNK) is a serine/threonine protein kinase of the AMPK family that plays pivotal role is several cellular processes such as cell survival, proliferation, and metabolism. [1,2]

HUNK expression is shown to be upregulated in several aggressive cancers including breast, ovarian, and colon cancers by supporting cell survival, resisting apoptosis, driving metastasis, and regulating tumor-associated macrophages.

HUNK is shown to phosphorylate its downstream target Rubicon at S92, promoting autophagy and tumorigenesis, driving survival and drug resistance in Her2+ breast cancers. [3,4] HUNK mediated cancer cell survival mechanism downstream of Her2/EGFR signaling and Akt activation, supports survival in presence of Her2 inhibitors.

In Triple negative breast cancers (TNBC) HUNK is shown to regulate tumor microenvironment supporting migration, as well as HUNK phosphorylation of EGFR T654 is shown to stabilize the receptor and increases cell migration and invasion. [5,6]



Growing literature supports HUNK as a potential therapeutic target against aggressive breast cancers, with only few inhibitors reported in literature. Here we screen a mini library of FDA approved drugs to identify compounds inhibiting HUNK phosphorylation activity. Interestingly, screening identifies Momelotinib, a recent FDA approved JAK1/JAK2/ACVR1 oral kinase inhibitor for treating intermediate or high-risk myelofibrosis with anemia, inhibits HUNK kinase activity with nM level of potency in biochemical assays. NanoBRET target engagement studies confirm Momelotinib engages with HUNK in cells. Momelotinib shows moderate inhibition of cell proliferation and activation of apoptosis in 4T1 and JIMT1 cell lines showing potential for this compound to be further evaluated as therapeutic for aggressive cancer cell lines, perhaps as combination therapy.

HotSpot Kinase Activity Assay Development

Recombinant full length human HUNK protein produced in insect cells were used for kinase activity assay development in Reaction Biology's HotSpot platform. HotSpot method is a miniaturized radiometric filter binding assay measuring ^{33}P labeled phosphate transfer from ATP to kinase substrate. HUNK activity assay was developed using Myelin Basic Protein (MBP) extracted from bovine as substrate.

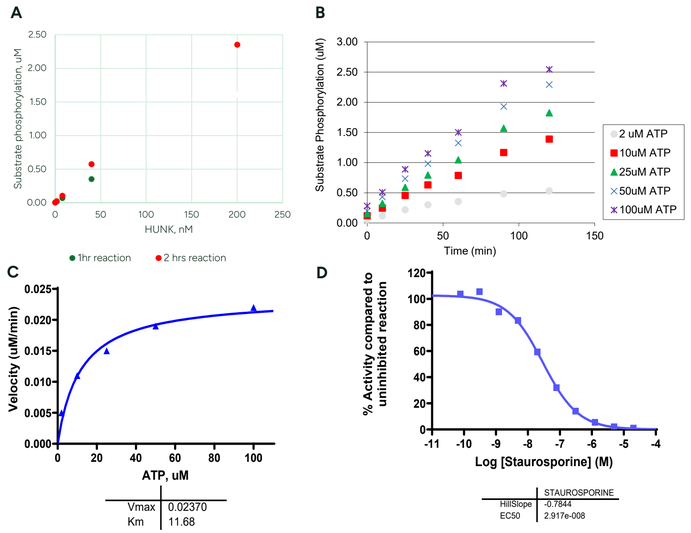


Figure 2: HUNK activity assay setup. A. Enzyme titration of HUNK enzyme at fixed concentration of ATP and substrate B. Substrate phosphorylation activity with time at varying concentrations of ATP and fixed concentration of enzyme C. ATP Km determination for HUNK D. Inhibition of HUNK enzyme activity by kinase inhibitor Staurosporine.

Evaluation of Momeltinib Activity in Cellular Studies

We performed cellular NanoBRET target engagement study with this compound to further evaluated Momelotinib activity with HUNK. HEK293 cells were transfected with 1 µg NanoLuc-HUNK Fusion Vector and 9 µg transfection carrier DNA. The transfected cells were treated with 10 doses of Momelotinib starting at 30 µM for 1 hour. Target engagement was readout by fluorescence ratio at 600nm/460nm in NanoBRET assay. Study confirms Momelotinib engagement with HUNK in cells.

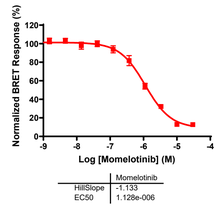


Figure 4: NanoBRET IC_{50} for Momelotinib vs HUNK

Momelotinib's effect in proliferation and apoptotic induction in HER2+ JIMT1 cells and triple-negative 4T1 breast cancer cells were evaluated. CellTiter-Glo studies show Momelotinib inhibited cell proliferation of both cell lines at high concentrations, while Caspase-3/7 activation assays indicated Momelotinib induces apoptotic cell death in both lines. These findings highlight Momelotinib as a potential therapeutic candidate for overcoming HER2 inhibitor resistance in breast cancer though HUNK inhibition.

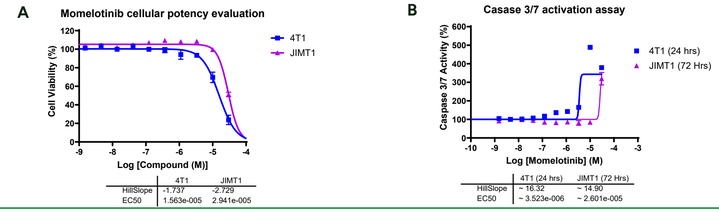


Figure 5: Effect of Momelotinib in proliferation of breast cancer cell lines 4T1 and JIMT1 A. CellTiter Glo assays and B. Caspase 3/7 activation assays

Conclusion

- A compound library of 144 FDA approved drugs was screened for inhibition of HUNK kinase activity in miniaturized filter binding kinase activity assay developed with recombinant HUNK kinase.
- Library screen identified Momelotinib, a JAK1/JAK2/ ACVR1 inhibitor inhibits HUNK activity in single digit nM potency.
- Cellular evaluation confirms Momelotinib engagement with HUNK in cells and apoptotic induction by Momelotinib in aggressive breast cancer cell lines 4T1 and JIMT1, showing potential of this compounds to be further evaluated in combination strategies against poor prognostic breast cancer types.

References

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FDA Approved Compound Library Screening for HUNK Inhibitor Discovery

Recombinant full length human HUNK protein produced in insect cells were used for kinase activity assay development in Reaction Biology's HotSpot platform. HotSpot method is a miniaturized radiometric filter binding assay measuring ^{33}P labeled phosphate transfer from ATP to kinase substrate. HUNK activity assay was developed using Myelin Basic Protein (MBP) extracted from bovine as substrate.

Compound ID:	% Enzyme Activity relative uninhibited reaction		Compound ID:	% Enzyme Activity relative uninhibited reaction		Compound ID:	% Enzyme Activity relative uninhibited reaction		Compound ID:	% Enzyme Activity relative uninhibited reaction	
Selumetinib (AZD6244)	108.60	108.68	Mobocertinib (TAK788)	106.32	98.88	Ponatinib (AP24534)	101.34	95.09	Protriptyline hydrochloride	91.05	104.34
Baricitinib (INCB028050)	86.35	74.22	Adagrasib (MRTX849)	107.45	108.24	Cabozantinib (BMS-907351)	109.58	98.15	Capivasertib (AZD5363)	90.96	92.21
Bortezomib (PS-341)	112.26	101.96	Pemigatinib (INCB054828)	102.05	104.79	Quizartinib (AC220)	104.22	106.57	Astemizole	96.84	95.93
Pyridoxine HCl	109.11	102.14	Pirtobrutinib (LOXO-305)	103.75	101.96	Cetirizine DiHCl	101.04	100.60	Samotolisib (LY3023414)	93.42	94.01
Vorinostat (SAHA)	108.11	93.84	Infigratinib (BGJ398)	100.34	97.07	Quizartinib (AC220)	93.77	100.72	Tucatinib	105.29	98.15
Mitapivat	105.38	97.69	Paxalisib (GDC-0084)	108.19	100.85	Azelastine HCl	99.99	100.97	Levocetirizine	103.66	98.47
Decitabine (NSC 127716)	108.20	104.73	Rabusertib (LY2603618)	98.43	92.30	Phenylephrine (NCI-C55641) HCl	103.07	98.92	Ritlecitinib (PF-06651600)	101.03	100.90
Tepotinib (EMD 1214063)	108.11	97.11	umbralisib (TGR-1202)	96.60	93.98	Resveratrol (SRT501)	92.26	91.85	Mavoraxfor	99.32	94.22
Tivozanib (AV-951)	97.32	86.63	GW3965 HCl	112.85	111.04	Brompheniramine hydrogen maleate	102.46	102.13	Desloratadine	101.29	95.95
Pexidartinib (PLX3397)	98.12	99.93	Tucatinib	100.63	105.46	Tirbanibulin	102.16	103.89	Momelotinib (CYT387)	2.09	3.43
Baricitinib (INCB028050)	78.81	73.24	Tirbanibulin	102.42	99.89	Chlorpheniramine Maleate	99.65	109.85	Hydroxyzine 2HCl	100.22	103.15
Tucatinib	100.80	101.86	Erdafitinib (JNJ-42756493)	105.21	99.11	Doxepin HCl	104.46	109.82	Amfebutamone (Bupropion) HCl	100.94	96.64
Dabrafenib (GSK2118436)	107.47	96.33	Captopril	105.45	110.77	Everolimus (RAD001)	101.02	97.93	Terfenadine	101.67	102.48
Cobimetinib (GDC-0973)	102.89	106.31	Sorafenib (BAY 43-9006)	102.69	97.17	Ibuprofen (NSC 256857)	103.26	100.40	Olopatadine HCl	108.89	93.23
Acalabrutinib (ACP-196)	93.47	98.86	Idelalisib	106.69	99.68	Doxylamine Succinate	97.07	99.03	Imipramine HCl	102.05	100.97
Pazopanib	90.00	89.89	Loriatinib (PF-6463922)	102.62	96.83	Repotrectinib (TPX-0005)	89.28	102.20	Binimetinib (MEK162)	97.60	105.32
Axitinib (AG 013736)	91.12	92.51	Fostamatinib (R788)	87.14	92.51	Salbutamol	100.83	96.71	Deudextromethorphan (AVP-786)	97.10	103.10
Regorafenib (BAY 73-4506)	103.18	94.09	Gilteritinib (ASP2215)	82.96	85.67	Darovasertib (LXS-196)	95.61	96.97	Duvelisib (IPI-145)	99.44	100.63
Bendamustine (SDX105) HCl	97.67	102.32	Ibrutinib (PCI-32765)	99.01	98.68	Remibrutinib (LOU064)	101.12	102.46	Pirtobrutinib (LOXO-305)	102.76	102.82
Entrectinib (RXDX-101)	96.88	94.51	Sunitinib (SU11248)	47.02	39.28	Abemaciclib (LY2835219)	64.44	68.58	Canertinib (CI-1033)	90.99	87.15
Diphenhydramine HCl	96.82	100.88	Alectinib (CH5424802)	84.77	92.08	Inavolisib (GDC-0077)	95.50	100.27	Neratinib (HKI-272)	92.46	93.28
Venetoclax (ABT-199)	105.11	111.32	Erlotinib (OSI-774)	91.34	86.56	Repotrectinib (TPX-0005)	96.56	97.00	Vandetanib (ZD6474)	101.59	102.14
Pacritinib (SB1518)	63.64	60.70	Fedratinib (TG101348)	94.11	84.54	Cimetidine	99.78	97.87	Palbociclib	100.30	97.11
lenolisib (CDZ 173)	104.93	93.03	Asciminib (ABL001)	106.15	99.86	Amitriptyline HCl	93.92	93.47	Maprotiline HCl	96.73	88.27
Apatinib	108.44	104.97	Alpelisib (BYL719)	102.37	100.56	Clemastine (HS-592) fumarate	99.58	95.80	CarbinoxaMine Maleate	101.57	99.27
Ripretinib (DCC-2618)	102.01	93.88	Neratinib (HKI-272)	86.66	83.78	Fexofenadine HCl	101.13	106.58	Clomipramine HCl	95.87	95.18
Upadacitinib (ABT-494)	100.86	106.65	Defactinib (VS-6063)	78.51	80.39	Capsaicin(Vanilloid)	102.17	100.95	Triprolidine Hydrochloride	95.84	87.60
Abrocitinib (PF-04965842)	97.93	103.11	Gedatolisib (PKI-587)	99.48	104.10	Nortriptyline hydrochloride	97.45	96.38	Bilastine	95.13	86.84
Avapritinib (BLU-285)	103.90	101.35	Lazertinib	80.04	83.77	Desipramine Hydrochloride	99.21	94.21	Prexasertib HCl (LY2606368)	63.99	64.76
Zanubrutinib (BGB-3111)	98.29	109.32	Capmatinib (INCB28060)	115.99	122.70	Fruquintinib (HMPL-013)	100.95	98.40	Amoxapine	96.30	93.60
Deucravacitinib (BMS-986165)	103.87	103.16	Cetirizine DiHCl	100.56	101.33	lenolisib (CDZ 173)	112.48	105.64	Rosuvastatin (ZD4522) calcium	102.03	101.50
Selpercatinib (LOXO-292)	90.77	100.93	Dasatinib (BMS-354825)	104.29	93.28	Remibrutinib (LOU064)	99.64	95.16	Tenalisib (RP6530)	106.76	102.09
Pevonedistat (MLN4924)	100.00	103.68	Fluoxetine (Lilly 110140) HCl	109.49	104.76	Chlorpromazine	96.61	102.70	Nintedanib (BIBF 1120)	96.16	93.09
Futibatinib (TAS-120)	100.28	109.29	Omeprazole	106.18	100.30	Mecamylamine Hydrochloride	97.03	92.30	Vemurafenib (PLX4032)	79.39	75.96
Pralsetinib (BLU-667)	98.64	97.14	Crizotinib (PF-02341066)	97.83	102.27	Tovorafenib (MLN2480)	93.07	100.23	Dovitinib (TKI-258)	56.27	55.42
Nilotinib (AMN-107)	73.88	71.07	Norgestrel	97.52	95.31	Ceritinib (LDK378)	80.66	79.91	Loratadine (SCH29851)	90.72	82.68

Table 1: High throughput screening performed with 1 µM HUNK identifies Momelotinib as a potent HUNK kinase inhibitor. All studies were performed at 10 µM ATP.

Momelotinib was tested in dose response format to calculate IC_{50} of HUNK kinase activity inhibition. HSL119, a previously identified first in class HUNK kinase inhibitor was tested alongside. Momelotinib inhibits HUNK activity with 7.9 nM IC_{50} while HSL119 inhibits HUNK at 1.4 µM IC_{50}. HSL119 IC_{50} value obtained in this test is in line with previous literature values of this compound by various assay formats. Both compounds show lower potency when the assays were performed at high ATP indicating that the compounds compete with ATP binding to HUNK kinase. This data also suggests that the potency of compound may be lower in cellular environment with high ATP concentration.

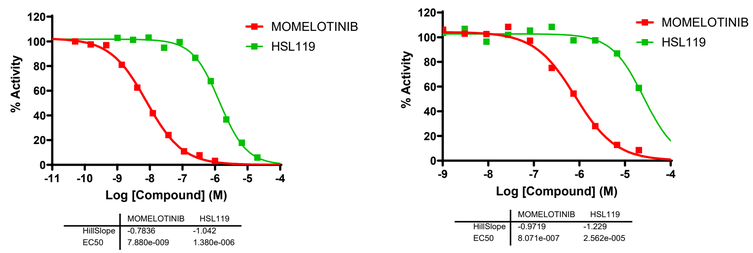

Figure 3: Biochemical IC_{50} value determination of Momelotinib and HSL119 in kinase activity assay A. at Km ATP concentration (10 µM) and B.at 1mM ATP concentrations.