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INHIBITION OF NFκB-INDUCING KINASE (NIK) SELECTIVELY ABROGATES NIK AND TRAF3 MUTANT MULTIPLE MYELOMA TUMOR GROWTH

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ABSTRACT

Enhanced NFκB signaling is a hallmark of aggressive lymphoid malignancies, including multiple myeloma (MM). Non-canonical NFκB signaling involves NIK-dependent activation of IKKα, which triggers nuclear accumulation of p52/RelB heterodimers. NIK is a highly unstable protein and degradation is mediated by a ubiquitin ligase complex consisting of TRAF2, TRAF3 and c-IAP1/2 (encoded by BIRC2/3). In a subset of MM, NIK is stabilized by mutations in NIK, TRAF2/3 or BIRC2/3. Here, we report on the first potent orally bioavailable NIK kinase inhibitor. TRC694 potently inhibits phospho-IKKα, prevents nuclear accumulation of p52/RelB (but not canonical NFκB) and represses the associated NFκB gene program selectively in MM cell lines with genetic activation of the non-canonical NFκB pathway. Proliferation of NIK translocated, TRAF3 or BIRC3 mutant MM cell lines is preferentially inhibited by TRC694 over MM cell lines which lack genetic activation of non-canonical NFκB. Consistently, elevated expression of a previously described 11-gene NFκB signature is predictive of sensitivity to TRC694 in MM. A single, oral dose of TRC694 to mice bearing a NIK-translocated MM tumor, inhibits phospho-IKKα, and represses p52-mediated transcription of NFκB regulated genes. Daily oral dosing of TRC694 completely inhibits expansion of NIK or TRAF3 mutant multiple myeloma tumors, with no signs of toxicities in these mouse models. In conclusion, TRC694 provides the first opportunity to test the clinical relevance of non-canonical NFκB inhibition in aggressive lymphoid malignancies.

BACKGROUND

NIK is Critical for Non-Canonical NFκB Activation

- **NFκB Inducing Kinase (NIK):**
  - essential for non-canonical NFkB (p52/RelB) activation
  - critical for B-cell maturation
- **NIK-deficient mice**:
  - viable, but reduced B-cell numbers
  - disrupted 2[ndary] lymphoid organ architecture
  - reduced Ig production
- **NIK activity**:
  - controlled through protein stability (TRAF2/3-cIAP1/2 E3 ubiquitin ligase)

Krappmann & Vincendeau, 2016

NIK is Activated in Aggressive B-cell Malignancies

- **Genetic alterations stabilizing NIK**, and activating non-canonical NFkB in B-cell malignancies:
  - **MM** –NIK, BIRC3 (cIAP2), TRAF2/3 (12-20%)
  - **MCL**–NIK, TRAF2/3 (17% at diagnosis, associated with Ibrutinib[R]), BIRC3 (10%)
  - **DLBCL**–TRAF3 (9-15%)
  - **cHL** –NIK (31%)
  - **CLL** –BIRC3 (4% at diagnosis, 24% in Fludarabine[R])
- **Ligand/receptor over-expression/amplification, and ligands** in bone marrow and lymph node can activate non-canonical NFkB
  - **MM** – ~50% of cases show activation of NIK pathway to the same extent as NIK translocated or TRAF3 mutant cases by NFkB gene signature
  - **DLBCL** – ~30% cases show activation of NIK pathway determined by nuclear p52 IHC
  - **cHL** – NIK stabilized in 55% of cases as determined by IHC
- **There are no therapies directly targeting non-canonical NFkB in clinical development**

Annunziata *et al.*/Keats *et al.*, Canc Cell 2007; Rahal *et al.* Nat Med 2014 ; Bea et al., PNAS 2013; Zhang *et al.*, Cell Rep 2015; Bushell et al., Blood 2015 ; Pham et al., Blood 2011; Rossi et al., Blood 2012; Ranuncolo et al., Blood 2012

This study was conducted by Janssen Research and Development

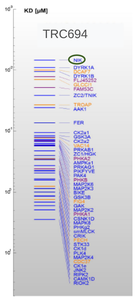
RESULTS

A. In vitro selectivity

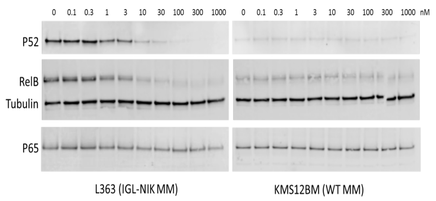
Pharmacology Summary TRC694

	Target Profile	TRC694
NIK kinase inhibition (IC_{50})	<10 nM	< 5 nM
NIK affinity and residence time (Proteros)	<10 nM Extended residence time (min)	< 5 nM > 100 nM
Kinase selectivity (DiscoverX)	<10% kinome at 100x NIK Kd	Kd (Nik) < 1 nM 32/409 kinases with 100x Kd
Cellular inhibition of pIKKα or pIkBα	<100nM pIKKα in L363 <100nM pIkBα in L363	< 5 nM < 10 nM
NIK/TRAF3 mutant MM/MCL 7day cell proliferation (median IC_{50})	<100nM (JJN3, MAVER1)	< 10 / < 10 nM
Selectivity window over WT NIK cell proliferation	>100-fold (KMS12BM, H929, TMD8, OCI-LY3)	>2000 / > 500 / >5000 / >5000 nM

Cellular Target Profiling in L363 cell lysates. Apparent affinities of TRC694 for various binding partners isolated from L363 cell lysates are displayed.

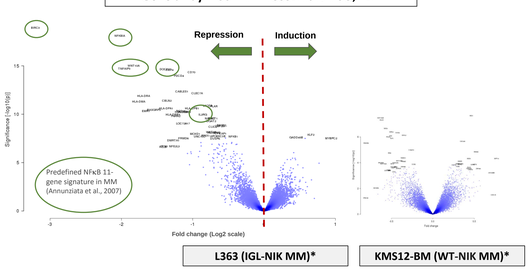


Potent inhibition of nuclear p52/RelB, but not canonical NFκB subunit p65 (RelA) in NIK translocated MM



TRC694 Preferentially Represses NFκB Genes in IGL-NIK

Gene array: 100 nM TRC694 vs DMSO, 24h



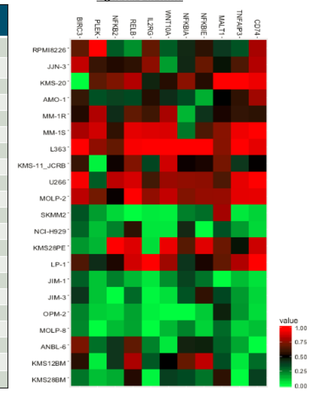
*drawn to same scale (Fold change/Significance)

Nuclear p52 correlates with TRC694 Sensitivity to the Same Degree as Mutation Status, or Activation of a MM NFκB 11-Gene Signature

TRC694, 7-day proliferation assay:

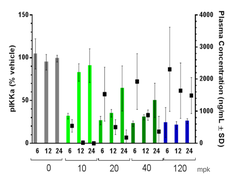
MM cell line	Relevant genotype	Median IC_{50} (nM)
RPMI-8226	TRAF3	4
JJN-3	EFTUD2-NIK	9
KMS-20	BIRC2/3	9
AMO-1	TRAF3	13
MM.1R	TRAF3	15
MM.1S	TRAF3	20
L363	IGL-NIK	23
KMS-11	TRAF3	44
U266	TRAF3	55
MOLP2	BIRC3	59
SK-MM-2	WT	79
NCI-H929	WT	214
KMS-28-PE	BIRC2/3	519
LP-1	TRAF3	526
JIM-1	WT	891
JIM-3	WT	990
OPM-2	WT	1579
MOLP8	WT	2115
ANBL6	TRAF3	4677
KMS-12-BM	WT	5849
KMS-28-BM	WT	10000

Expression of NFκB gene signature in MM:

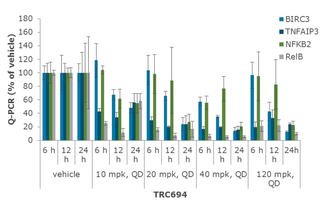


B. In vivo results

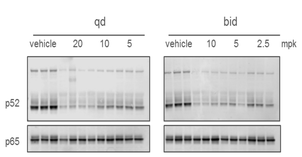
Potent inhibition of NIK and repression of p52-mediated NFκB genes upon oral dosing of TRC694 to NIK-translocated-tumor bearing mice



pIKKα inhibition and plasma compound (TRC694) exposure levels after a single oral dose of TRC694 in mice bearing JJN3 (MM) xenografts

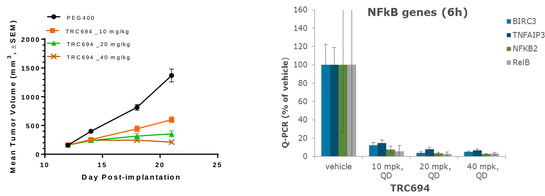


NFκB gene repression after a single dose of TRC694 in mice bearing JJN3 xenografts



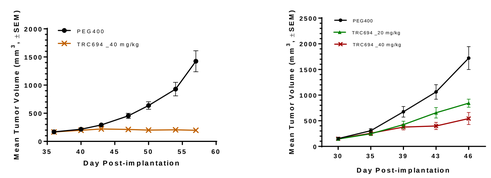
p52 nuclear exclusion after once daily (qd) or twice daily (bid) dosing of TRC694 for 3 days, in mice bearing JJN3 xenografts

TRC694 is Efficacious in the NIK-translocated JJN-3 Model



- Pre-established NIK-translocated MM model (JJN3). Once-daily (qd), oral dosing.
- Efficacy consistent with NFkB gene repression at end of study.
- Minimal efficacious dose is 10 mpk qd, maximal efficacy seen at >20 mpk qd (84,6% TGI with 40 mg/kg)

TRC694 is Efficacious in TRAF3 Mutant MM Models



- Pre-established xenograft model. Once-daily, oral dosing using PEG400 solution
- Significant efficacy seen in pre-established TRAF3 mutant xenografts after once daily oral dosing of TRC694 (RPMI-8226 : 88,2% TGI, MM.1S : 68,4% TGI)
- Efficacy observed (~50% TGI) in TRAF2 mutant Ibr[R] MCL model (Z-138)

CONCLUSIONS

- **Genetic alterations (NIK, TRAF2/3, BIRC2/3), leading to NIK protein stabilization and activation of non-canonical NFκB, are found in aggressive B-cell malignancies**
- **TRC694 is a potent and selective, first-in-class, orally bioavailable small molecule NIK inhibitor that inhibits the growth of B-cell malignancies with activation of the non-canonical NFκB pathway in vitro and in vivo**

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