# Emerging Indications for Kinase-Targeted Therapies: Screening for New Targets

Rick Lee, Shenshen Lai, Allan Mah, Hong Zhang, Jun Yan, **Anthony Marotta**, Zaihui Zhang
**SignalChem Lifesciences Corporation, 110 - 13120 Vanier Place Richmond, BC V6V 2J2;**

**Abstract No. A152**
**AACR-NCI-EORTC Meeting 2017**

## INTRODUCTION

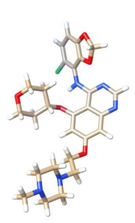
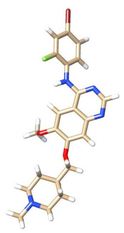
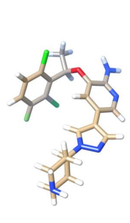
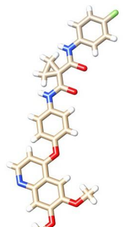
- Targeted small-molecule kinase inhibitors (SMKIs) have vastly led to improved clinical outcomes, mainly in oncology/rheumatology.
- Currently there are more than 30 FDA approved SMKIs.
- Evaluating off-target effects across the kinome, may provide insights into evaluating efficacy of drugs for other clinical indications.
- SignalChem has over 800 wild type and mutant protein kinases and offers profiling services for most of these targets.

## STUDY OBJECTIVE

In this study, we evaluated the specificity of four known SMKI agents across a selected panel of 48 kinases from eight sub-families at two concentrations with intent to identify off target effects and to define new areas to exploit these drugs.

## METHODS

**Table 1. SMKIs used for kinase panel screening**

| | Saracatinib | Vandetanib | Crizotinib | Cabozantinib |
|---|---|---|---|---|
| **Structure** |  |  |  |  |
| **Formula** | $C_{27}H_{32}ClN_5O_5$ | $C_{22}H_{24}BrFN_4O_2$ | $C_{21}H_{22}Cl_2FN_5O$ | $C_{28}H_{24}FN_3O_5$ |
| **CAS Number** | 379231-04-6 | 443913-73-3 | 877399-52-5 | 849217-68-1 |
| **Primary Target(s) / $IC_{50}$(s)** | SRC / 2.7 nM<br>Bcr-Abl / 30 nM | KDR / 40 nM<br>FLT4 / 110 nM | MET / 11 nM<br>ALK / 24 nM<br>ROS1 / 0.11 nM | KDR / 0.035 nM<br>MET / 1.3 nM |
| **Other Known Targets / $IC_{50}$(s)** | LCK / <4 nM<br>YES / 4 nM<br>LYN / 5 nM<br>FYN, FGR, BLK | EGFR<br>HER2<br>RET | | KIT / 4 nM<br>AXL / 7 nM<br>RET / 4.6 nM<br>TIE2 / 14.3 nM |
| **Therapeutic Area(s)** | Cancer, Alzheimer's Disease | Cancer | Adenocarcinoma, Lymphoma, GBM, Colorectal, Breast, HCC, Urothelial, Melanoma | Thyroid Cancer, Renal Cell Carcinoma |
| **Approval Status** | Discontinued | Approved for Thyroid Cancer | Approved for EML4-ALK positive Lung Cancer | Approved for Medullary Thyroid Cancer |

## PROTEIN KINASE PANEL DETAILS

**Table 2. Panel Kinases and Their Subclasses**

| Kinase Subclass | Brief Description | Kinases in Panel |
|---|---|---|
| **Receptor TK (RTKs)** | RTKs are cell surface receptors that promote cell growth, differentiation, migration and survival. | ALK, ALK1, ALK2, AXL, c-KIT, DDR2, EGFR, EPHA1, EPHB1, FGFR1 (FLT2 FLT1, FLT3, HER2, InsR, MET, PDGFRb, TIE2, TRKA |
| **Cytoplasmic TK (CTKs)** | Several of the larger CTK subfamilies include the SRC, JAK, TEC and ABL kinases. | ABL1, BTK, FAK, FER, KDR, LCK, SRC ZAP70 |
| **CAMK** | CAMKs mediate second messenger effects of $Ca^{2+}$, with many acting as metabolic sensors. | AMPK (A1/B1/G1), MAPKAPK2, MLCK |
| **CMGC** | CMGC kinases target proline-rich target sequences. CDKs are important members with essential roles in cell cycle regulation. | CDK2/CyclinA2, ERK1, GSK3 beta |
| **AGC** | AGC kinases share similarities within their catalytic kinase domain. | p70S6K, PKC alpha, ROCK1, RSK1, SGK1 |
| **STE** | The STE subfamily include many enzymes involved in MAP kinase signaling. | MST1 |
| **TKL** | TKLs resemble tyrosine kinases and they include IRAKs and RIPKs. | BRAF, IRAK2, LRRK2, MLK3, RIPK1 |
| **Other** | This class includes kinases that are not classified in conventional subfamilies. | AURORA A, CK2 alpha 1, IKK alpha, NEK2, PLK1, ULK1 |

## UNIQUE AND OVERLAPPING TARGETS

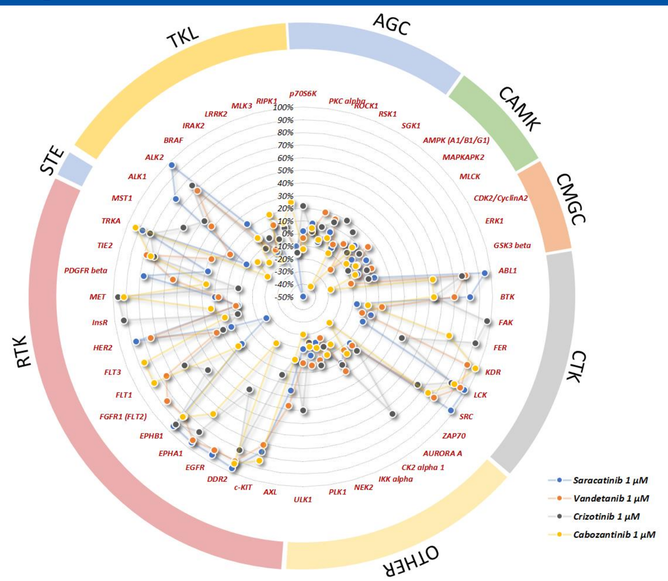



**Figure 1. SMKI profile comparisons at 1 µM.** Saracatinib, vandetanib, crizotinib and cabozantinib were tested at 1 µM; with % inhibition as the radial axis. As each SMKI represented ATP competitive tyrosine kinase inhibitors, the profiles shared much overlap. However, several unique 'hits' were identified, such as crizotinib's ability to inhibit Aurora A. Additionally, many off-target kinases were observed, which include saracatinib's ability to inhibit EGFR and HER2, members of the epidermal growth factor receptor family of RTKs.

## ESTIMATING [TARGET] RANGES

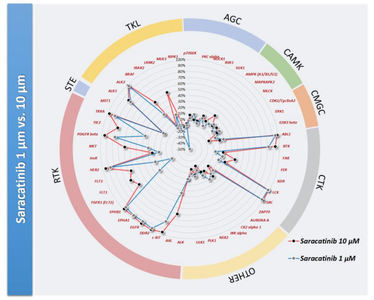



**Figure 2. SMKI concentration vs. inhibition profile.** 1 µM and 10 µM profiles of saracatinib, vandetanib and crizotinib % inhibition were plotted on the same axes, which can help to pinpoint target ranges for follow-up $IC_{50}$ studies.

## SUMMARY

- All tested SMKIs are ATP competitive inhibitors
  - The compounds have overlapping profiles
  - "Hits" are predominantly tyrosine kinases
- Known targets of saracatinib (SRC, ABL and LCK) were confirmed at both concentrations.
  - "Hits" are predominantly tyrosine kinases
  - 1 µM and 10 µM SMKI treatments resulted in similar target specificity
- Dose-dependent changes were observed in vandetanib targets EGFR and HER2 along with VEGFR2/3-related enzymes.
  - Vandetinib's profile has significant overlap with saracatinib, with ABL1, LCK and SRC displaying dose-dependent inhibition
- Crizotinib has the most divergent inhibition pattern, also inhibiting the activity of TKL members ALK1/2, the STE kinase SMT1 and several members of the AGC-class at higher concentration.
  - Although crizotinib acts as a mild inhibitor of EFGR at 1 µM, at 10 µM, it acts as an activator, increasing activity by almost 50%
  - Crizotinib appears to be a potent inhibitor of InsR, almost eradicating activity at 1 µM, opening possible implications for regulating metabolic homeostasis.
- The inhibition profile of Cabozantinib overlaps with that of vandetanib and saracatinib, inhibiting a broad selection of RTKs, including EGFR along with cytoplasmic TKs, such as SRC and LCK.