Clayton Biotechnologies

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YESS-KI:
Predicting Kinase Inhibitor Resistance

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YESS-KI: developing predictive biomarkers

- YESS-KI is a technology that produces reliable predictive biomarkers for cancer therapy.
- While incredible progress has been made in treating cancer, there remains an unfortunate high level of treatment failure.
- Drug development efforts have been plagued with very low success rates, especially with regards to cancer therapeutics. The New York Times reported figures from a recent study showing that *"only 3 percent of cancer drugs tested in clinical trials between 2000 and 2015 have been approved to treat patients."*
- The use of patient selection biomarkers significantly increased success rates in drug development. For overall drug development efforts (all indications), success rates without biomarker usage were reported at 8.4% while with the use of biomarkers, this number increased to 25.9%.
- But identifying a reliable and predictive biomarker is difficult
- *YESS-KI is an inexpensive, efficient and reliable platform to accompany drug development from early pre-clinical stages to patient stratification in clinical testing.*

Kinase Inhibitors (KI).

- KIs are an extremely effective class of therapeutic drugs and due to their specificity are associated with low side effects.
- Cancer patients often require chronic KI treatment, and unfortunately many experience resistance and intolerance, necessitating a change to 2nd or 3rd line KI therapy.
- **Prescribing the correct course of therapy** is difficult, ***especially faced with an increasing choice of KIs***. Question are raised such as:
  - *Will the patient's disease respond to the new treatment?*
  - *Which therapy going forward will be most effective?*
  - *Will the new therapy also result in resistance or intolerance?*

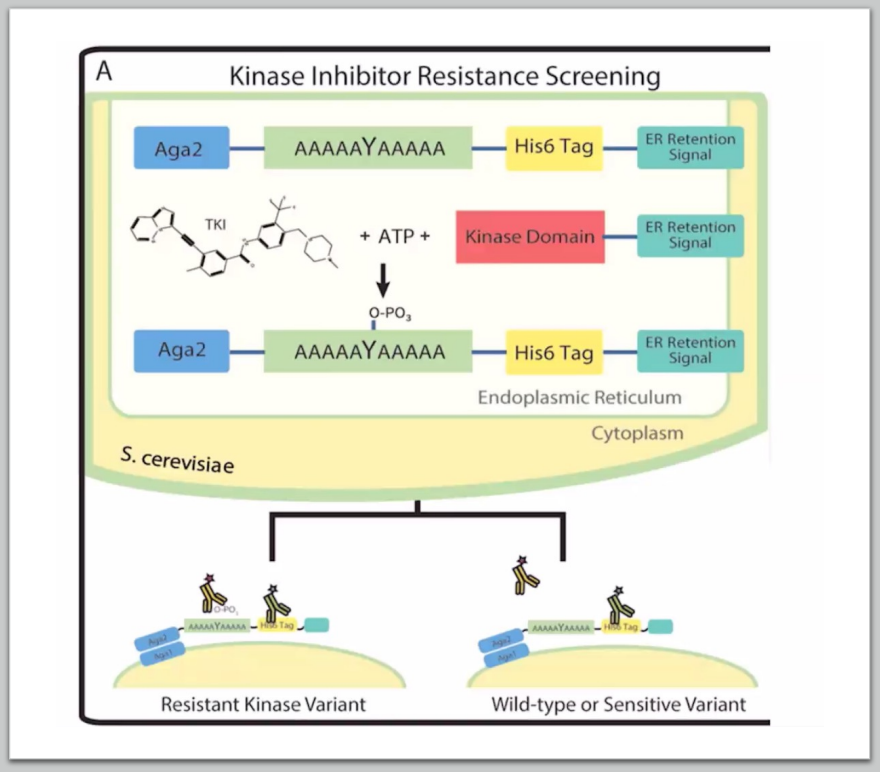
Kinase Inhibitors (KI).

- With over 60 FDA approved KIs with hundreds in development, patients have many good options.
- And with so many options, the hope is that an effective KI treatment will match each patient.
- **However, we will need tools to predict which KI will work for a specific patient.**
- Current methods for predicting resistance are slow, costly, and not comprehensive.
- **Doctors and drug developers** need better tools to select patients with a high likelihood of responding to treatment.
- KI pre-clinical candidates need better models to predict and translate their outcomes for patient use.

PRESS

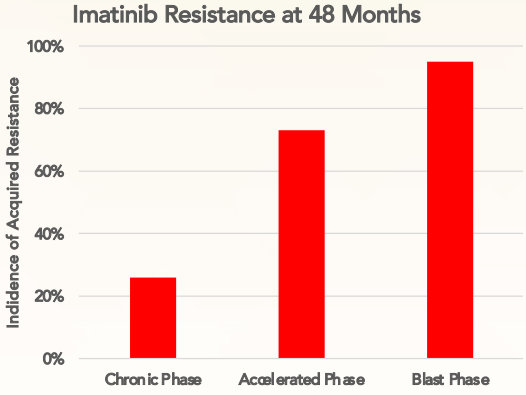
Assay to predict drug resistance.

- **Our solution: YESS-KI, a low-cost yeast-based assay to predict resistance mutations to KIs.**
- Allows selection of KI therapy with fewer modes of resistance.
- By predicting the resistance of a kinase inhibitor, we can predict whether a drug will work for a given patient and thereby improve outcomes for patients.
- Also allows for creation of drug vs mutation matrix to help oncologists choose appropriate KI for given mutational landscape.



Imatinib: A First-in-Class Cancer Therapy.

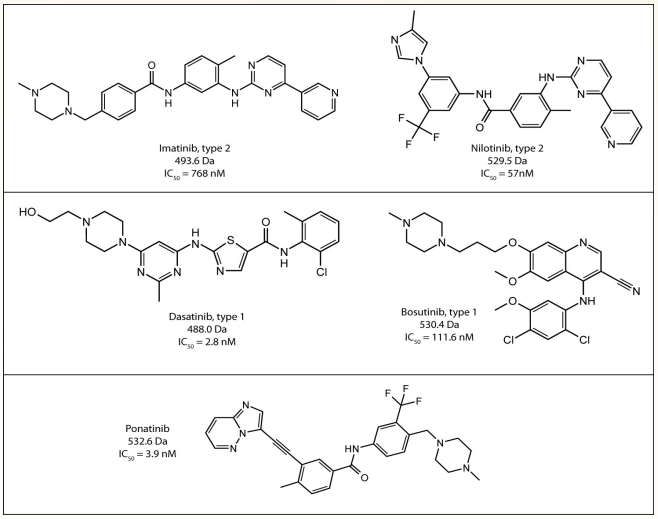
- First approved small molecule targeted cancer therapeutic
- Inhibits the BCR-ABL oncogene (a tyrosine kinase), and results in drastic increase in survival.
- However, acquired resistance in a large number of chronic-phase patients, leads to treatment failure.
- This has led to development of second- and third-generation BCR-ABL inhibitors.
- *Will these new 2nd and 3rd generation drugs overcome resistance mechanisms and provide better outcomes for patients on chronic therapy?*



Data from Shah, NP. Hematology Am Soc Hematol Educ Program. 2005

Next-Generation BCR-ABL Inhibitors have been developed and implemented in the clinic.

- Second-generation:
  - Nilotinib
  - Dasatinib
  - Bosutinib
- Third-generation:
  - Ponatinib
- Fewer resistance mutations
- Increased affinity



YESS-KI predicts acquired resistance in Bcr-Abl.

➢ *Does our YESS-KI assay accurately predict patient resistance to KI treatment? We compared dasatinib and ponatinib:*

Dasatinib:

- ➢ All known dasatinib-resistant mutations were seen with our test.
- ➢ Of our top 5 most common dasatinib-resistant mutations, four have been previously reported.

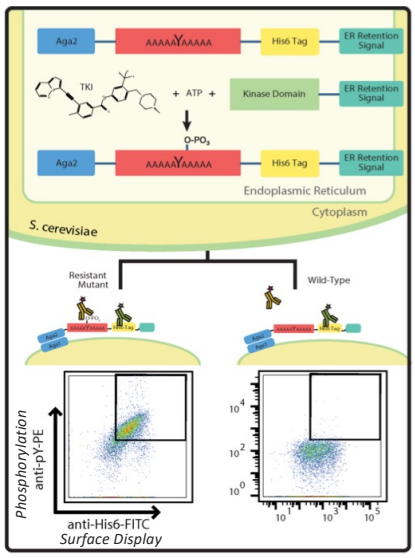
Ponatinib:

- ➢ Top mutant, E255V, is the most resistant single mutation isolated from patients.
- ➢ Ponatinib-resistant clones were enriched for compound (2+) mutations, which have been observed in patients and validated in vitro.
- ➢ IC50 of novel mutations measured by cell culture to validate results in YESS.

YESS-KI Resistance Assay – how does it work?

- From an initial population of random mutants, inhibitor-resistant mutations are enriched by cell sorting.
- Screened and initial libraries are sequenced.
- Mutations are identified and compared to known data, if available.
- Novel mutations are assayed first individually in the YESS-KI system, then validated by previously established in vitro methods.
- Our libraries cover the entire single mutation space as well as most double mutations

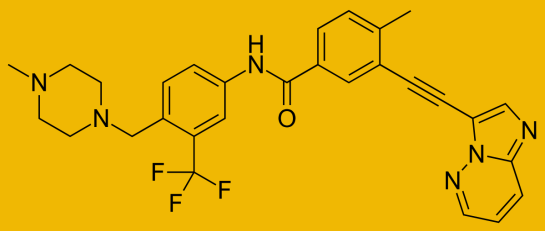
THE MECHANISM

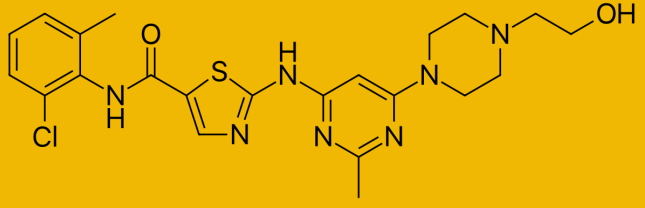


YEAST - FACS - NGS

YESS-KI correctly predicted acquired resistance.

- We found EVERY ONE of the mutations seen in the clinic for both **dasatinib and ponatinib** (as well as some not yet seen).
- We showed that **ponatinib** activity required the much more rare situation of two mutations to see resistance.
- Resistance to **dasatinib** was seen with single mutations.
- **YESS-KI predicts that Ponatinib is a better drug with less resistance**, even when used in long-term treatment.
- We could have predicted the futures of dasatinib vs. ponatinib in the clinic.



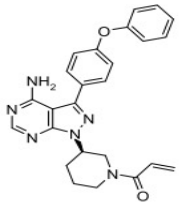


Ibrutinib and Alacalbrutinib are irreversible KIs.

- *BTK inhibitors used to treat B-cell lymphomas and leukemias.*

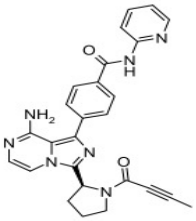
IBRUTINIB:

- In 2018 Ibrutinib earned $3.6 billion in for AbbVie
- Projected sales for 2020 are $5 billion/year
- Broad specificity (inhibits 8 other kinases)
- 16-21% of patients acquire resistance

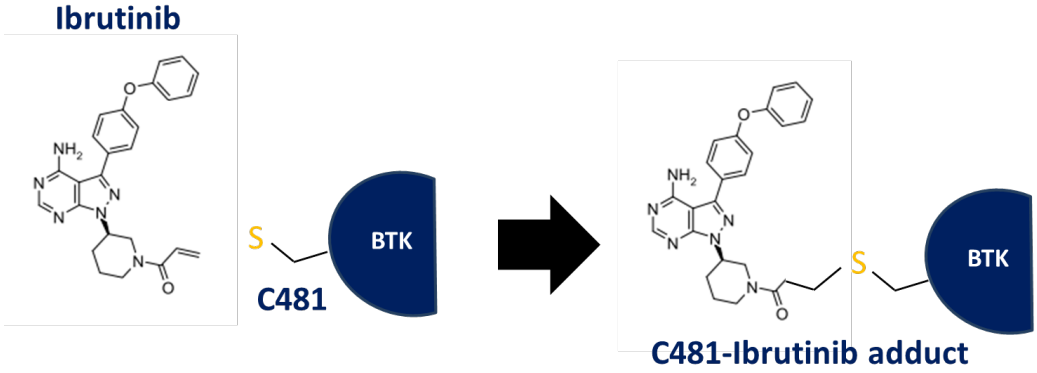


ACALABRUTINIB:

- Approved in October 2018 for mantel cell lymphoma.
- Less cross-reactivity than Ibrutinib.



C481S is most common resistance mutation for Ibrutinib and Acalabrutinib.



- **C481S mutations block KI binding irreversibly.**

YESS-KI predicts acquired resistance in BTK.

- *Does our YESS-KI assay accurately predict patient resistance to KI treatment?*

Ibrutinib:

- Two most prevalent clinical resistance mutations found: C481S and T474I.
- Additional mutations that alter "DFG" in confirmation found.

Acalabrutinib:

- C481S among top resistance mutations.
- Several new mutations discovered that may alter stabilization of the C-α Helix.

We also identified previously unreported resistance mutations and validated in vitro.

YESS-KI predicts acquired resistance and proposes alternative KIs

- **Screening multiple inhibitors in YESS-KI will allow us to create a rubric of inhibitors and their efficacy against resistance mutations as a prognostic tool for oncologists.**

Profile for BTK inhibitors:

	WT	C481S	Mutant 2	Mutant 3	Mutant 4
Acalabrutinib	+	-	-	-	-
Ibrutinib	+	-	+	+	-
Vecabrutinib	+	+	+	-	+
Remibrutinib	+	-	+	+	-

YESS-KI is efficient, scalable, and broadly applicable.

- From screening to data analysis, YESS-KI can be completed in less than two weeks.
- YESS-KI can be applied to combination treatment or sequential treatment with kinase inhibitors to simulate treatment regimes.
- YESS-KI can be performed in parallel for comparisons between inhibitors.
- **Several kinases and multiple inhibitors have been shown compatible with YESS-KI system.**
  - Kinase Inhibitors tested: imatinib, dasatinib, ponatinib, gefitinib, and Osimertinib
  - working on datasets for infigratinib, futibatinib, erdafitinib, pemigatinib.
  - generated libraries to several kinases (Src, Lyn, Abl, BTK, Bcr-Abl)
  - making and testing libraries for FGFR2, Her2 and Alk

Summary: YESS-KI to predict resistance to KIs.

- <u>Diagnostic</u>
  - **YESS-KI provides predictive biomarkers to determine the best KI treatment for a patient.**
  - **The rapidly expanding number of approved KI's have created the critical need for diagnostic tools capable of rapidly identifying which new drug is most appropriate for an individual patient**
- <u>Drug Development</u>
  - We offer a yeast-based assay to discover resistance mutations for approved and candidate kinase inhibitors (KIs).
  - YESS-KI represents a tool for drug developers:
    - To efficiently (under 2 weeks) select the best drug candidate in pre-clinical development
    - For patient stratification in clinical trials

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Thank you for your attention