AMGEN
Pioneering science delivers vital medicines™

Development of Fit-for-Purpose Customized Assay Statistical Tools (CAST) for Immunogenicity Assays

Cheng Su[1], Lei Zhou[1], Ning Weng[1], Yu Wu[1], Naren Chirmule[2], Zhandong Don Zhong[2]

1: Research & Translational Sciences Biostatistics, Amgen Inc.

2: Clinical Immunology, Amgen Inc.

Outline

- Introduction of CAST
- Immunogenicity testing and Assay Cut Point
- The automated system
- Fit-for-purpose qualification test
- Conclusion

Customized Assay Statistical Tools (CAST)

Web base systems developed to automate specific types of repetitive experiments and intended to be used by scientific colleagues

- User friendly
  - Built-in statistical decision making
  - Outputs are report-ready
- Customization
  - data input format, analysis and output
  - user Interface
- Gain efficiency, ensure quality with reasonable system development cost

CAST: Meeting Statistical Needs in Resource Limited Environment

1. **Statistician hands-on analysis**

Statisticians and scientific partners work closely together on individual studies

Design, analysis, report, interpretation

Pros: highest quality

Cons: FTE, turnaround time, subjectivity

3. CAST

Analysis and reports customized and automated for repetitive studies

Pros: quick, better quality and more flexible than 2; provides standards

Cons: only cost effective for repetitive studies, may fail on anomalies

2. **Commercial Software**

Scientific partners use software with/without training from statisticians

Pros: no statisticians FTE required

Cons: difficult for complex analysis, no quality control

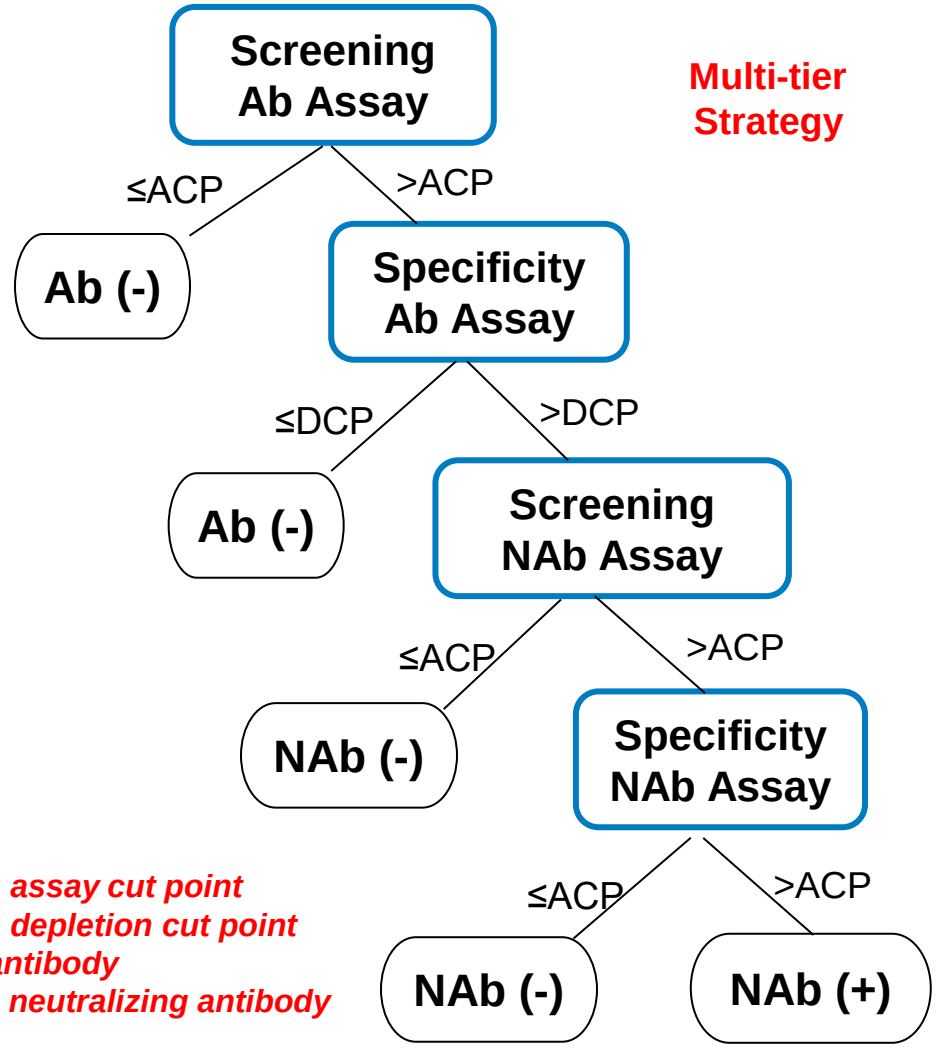
To achieve significant efficiency gain with minimum quality loss

Immunogenicity Testing Strategy

Importance

- Immunogenicity may affect drug exposure, efficacy and safety

- Immunogenicity assessment affects many areas
  - Preclinical Development
  - Clinical Development
  - Regulatory Affairs
  - Medical Affairs
  - Sales & Marketing



Statistical Applications for Immunogenicity Assay Validations

- Assay cut point
- Precision and accuracy
- Non-linear curve fitting
- Assay sensitivity
- Assay acceptance criteria
- Reagent equivalence test
- Proficiency test
- Assay transfer

CAST for Immunogenicity Assays Analysis

- Relieve resource constraint, reduce data turn-around time, and minimize subjectivity in data analysis
  - Data analysis not trivial
  - Repetitive, but has multiple steps
  - Versatile experimental designs
  - Current commercial software lack customization and require statistical decision making
- Standardized and automated data analysis and reporting
- Achieve greater compliance with a **qualified, fit-for-purpose** system

Statistical Background on ACP Estimation

- ACP is a one-sided 95% prediction limit
- Base on assay validation experiment
- Ensure normality: outliers removal and data transformation
- Mixed effect modeling
  - Full model -> reduced model
  - Variance Component Analysis
- Calculation of prediction limit
  - Total variance
  - Degrees of freedom approximation

Standard Experimental Design and Model Library

- Every design is matched with a (full) model
  - Consider all factors random effects
- Current system allows seven models
  - It is easy to add more designs

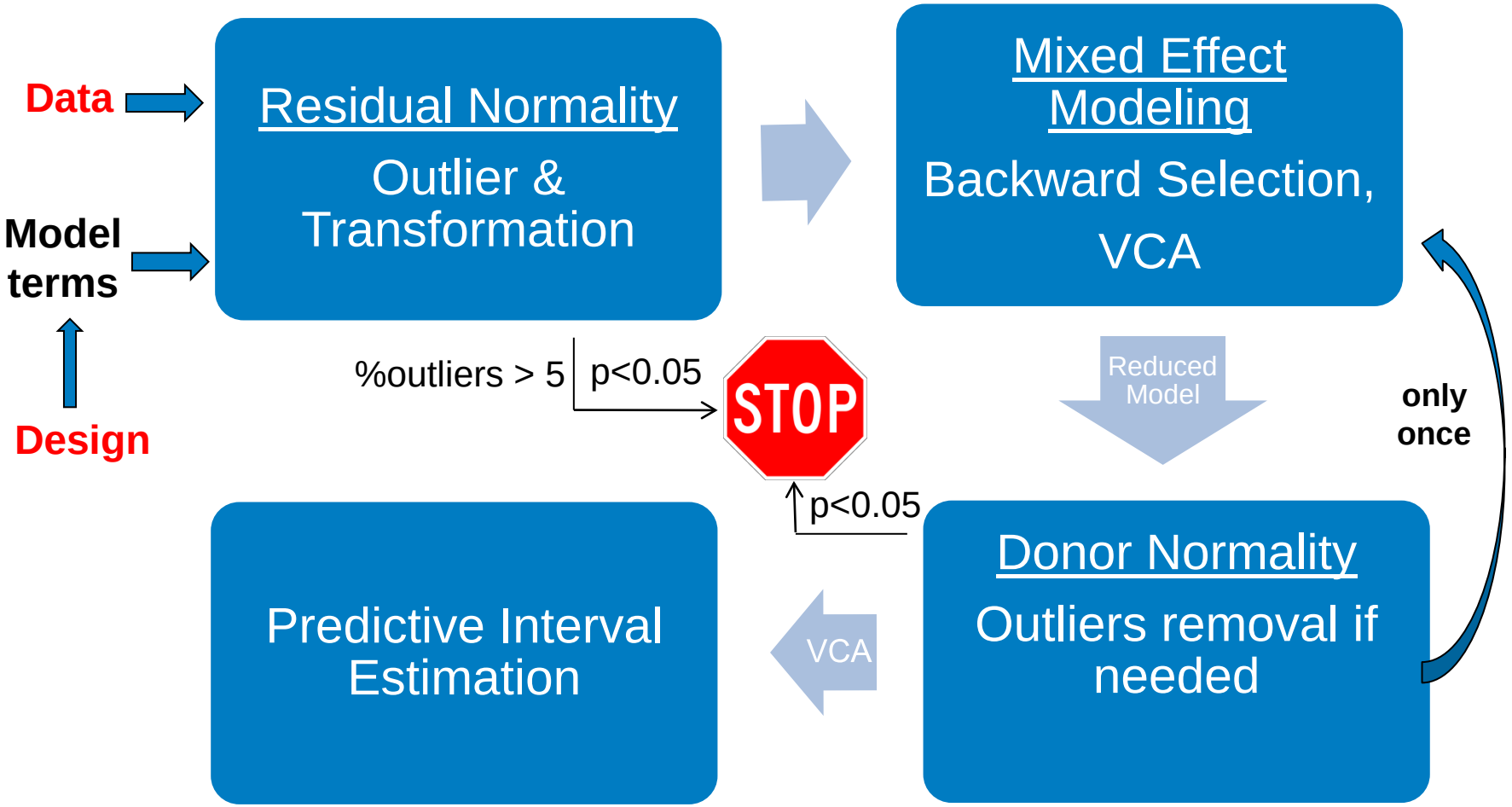
e.g., Bioassay Design 2

Drug	Assay Run	Analyst	Date	Plate(s)	Random Sequences
xxx	1	1	1	2	
		1	2	2	S1,S2, S3,S4,
		1	3	≥2	S5,S6, …...
	2	2	4	2	
		2	5	2	S1,S2, S3,S4,
		2	6	≥2	S5,S6, …...

- Donor samples randomized into six sets (S1, S2, …, S6)
- Model:

Analyst + Date within Analyst + Plate within Analyst and Date + Donor

Analysis Work Flow in CAST

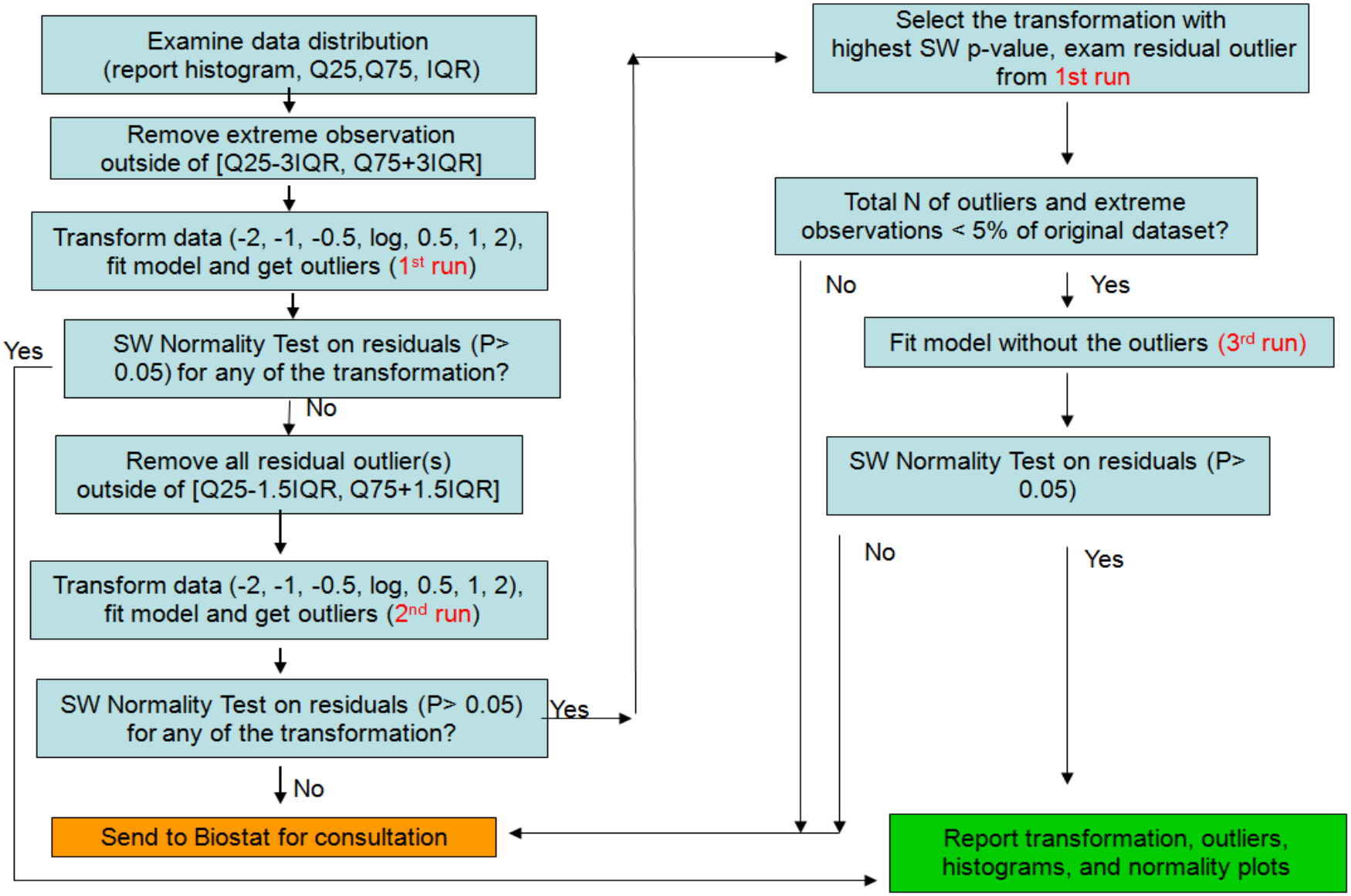


AMGEN

Challenges

- Prove that automation is feasible
- Maintain quality and ensured proper use
- Meet compliance requirements
- Changes in experimentation

Data Transformation and Outlier Removal Module



Predictive Interval Calculation

Cut point = LS-mean + TINV(.95, DF)*SQRT(VarTotal + Var(LS-mean))

- VarTotal (VT) = $V_1 + V_2 + \ldots + V_n$, V_i is the variance component
- V_i is estimated from Expected Mean Square Table

e.g.

Source	DF	MS	EMS
DONOR_ID	59	0.013841	Var(Residual) + 0.5822 Var(PLATE(Run)) + 4 Var(DONOR_ID)
PLATE(Run)	9	0.036162	Var(Residual) + 20 Var(PLATE(Run))
Residual	171	0.002384	Var(Residual)

Df ***MS*** **X** ← Put the coefficients into X matrix

- Method of moment: $\mathbf{X} * \boldsymbol{V} = \boldsymbol{MS} \Rightarrow \boldsymbol{V} = X^{-1} * MS$

  Hence, VT = $\boldsymbol{a'} * X^{-1} * MS$, let $\boldsymbol{a'}$ be a row vector of 1

- DF of VT based on Satterthwaite's approximation:

$$DF = VT^2 / [(\boldsymbol{C}^2 * \boldsymbol{MS}^2) / \boldsymbol{Df}],$$

where $\boldsymbol{C} = \boldsymbol{a'} * X^{-1}$, coefficients of ***MS*** to VT

and Var(LS-Mean) is negligible

Implemented in SAS IML

Maintain Quality and Ensure Proper Use

- Extensive comparison of CAST-ACP and manual result
- System defers to Biostatistician in certain conditions
- Standardization to reduce potential error
  - Data templates
  - Standard designs

- Statistician review of critical results
- User manual, SOP, training, e-learning and certification

Automation vs. Manual Analysis (Immunoassay)

MSD

Assays	Statistical Methods	Transformation Power	U95 bound	#Outlier
1	Manual	1	1.10	0
	CAST	-1	1.11	0
2	Manual	1	1.11	2
	CAST	1	1.20	3
3	Manual	1	1.10	4
	CAST	-2	1.13	7

Biacore

Assays	Statistical Methods	Transformation Power	U95 bound	#Outlier
4	Manual	0.5	58.80	0
	CAST	0.5	58.80	0
5	Manual	Poor data distribution		
	CAST	**Could not find transformation, send data to biostat**		

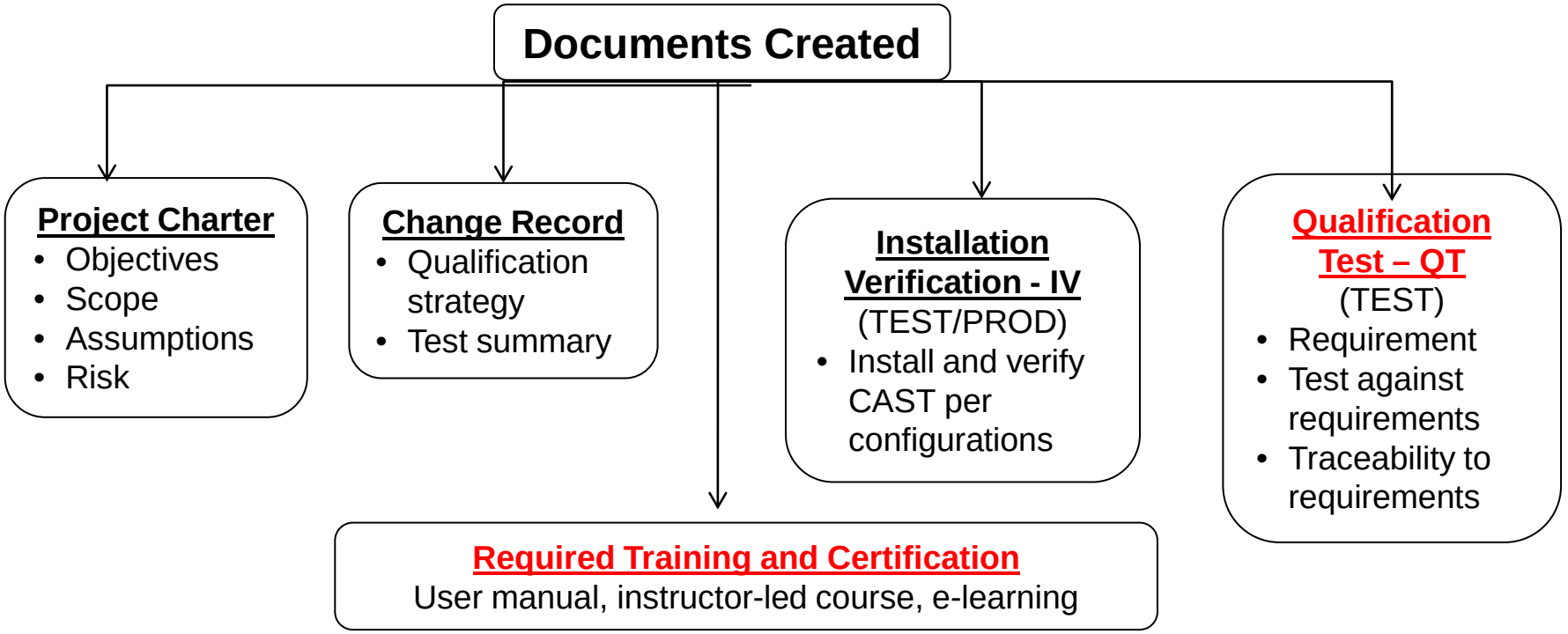
Automation vs. Manual Analysis (Bioassay)

Bioassay

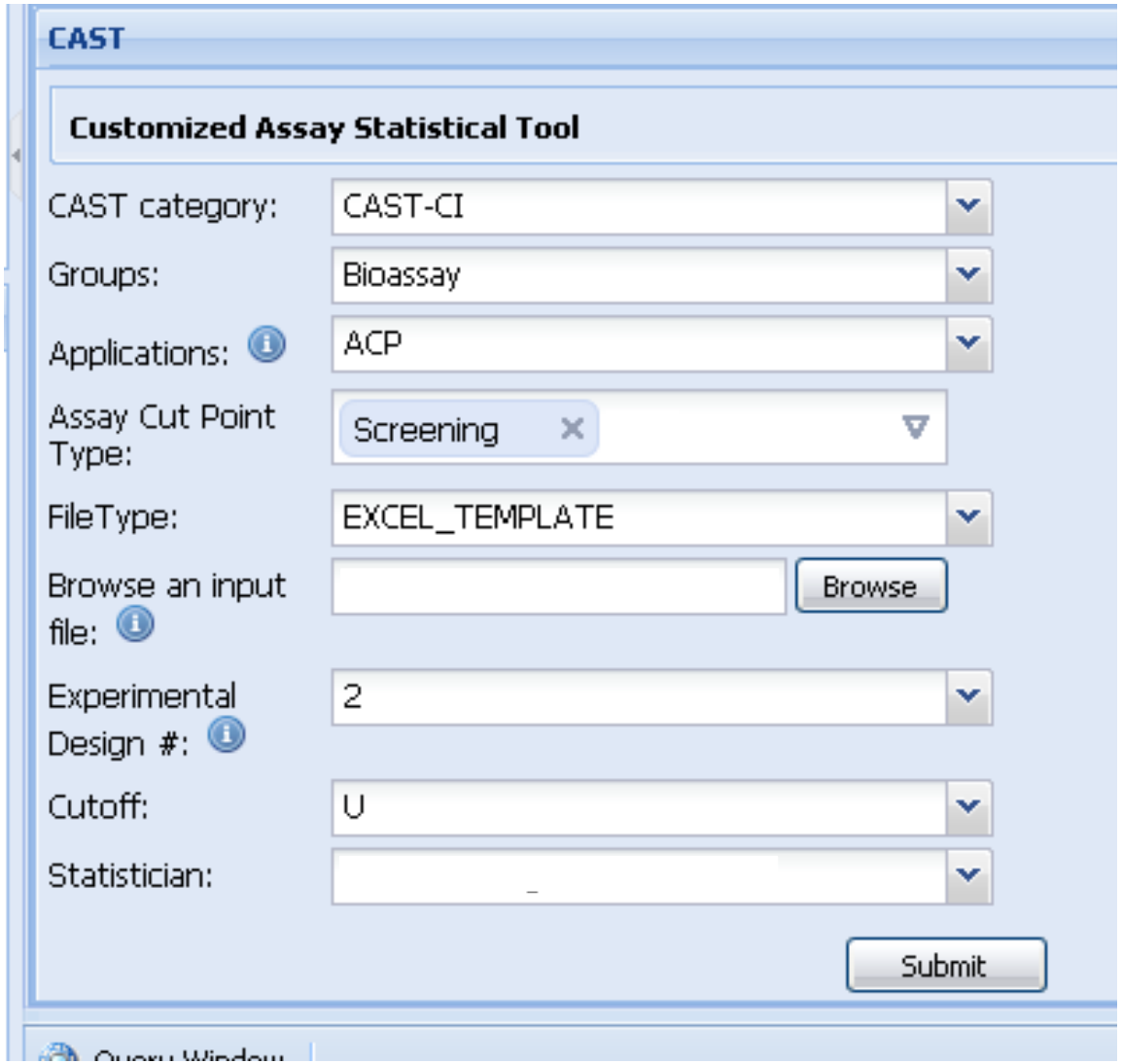
Assays	Statistical Methods	Transformation Power	U95 bound	#Outlier
6	Manual	1	2.68	1
	CAST	-2	2.76	1
7	Manual	1	1.88	2
	CAST	2	1.64	1

Fit-for-purpose Qualification and Compliance

- Good Software Engineering and Good Documentation Practices, Change Control, Qualification Test
- Trainings, certification and statistician signed off



CAST ACP Web User Interfaces- Input

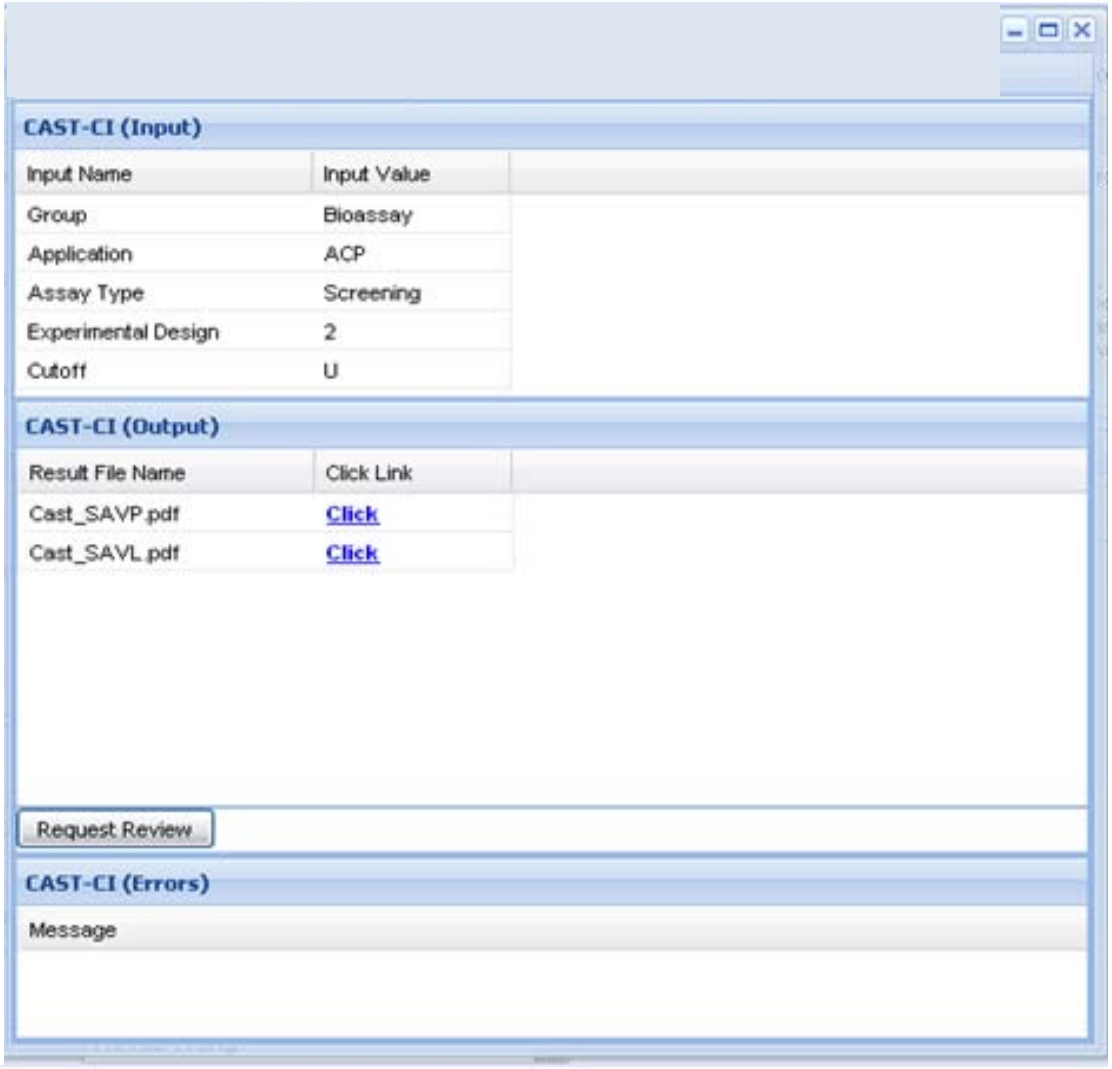


Interface

- **2 groups**
  - **Immunoassay**
  - **Bioassay**
- **3 types of cut points**
  - **Screening, Specificity, Scr/Spec**
- **2 input Data Format**
  - **Excel**
  - **Files from instrument**
- **7 experimental designs**
- **Upper/lower bound**
- **0 statistical decision**

AMGEN

CAST ACP Web User Interfaces - Result



- Analyzed by SAS on a server
- Click to get PDF outputs with unique id for traceability
- Outputs also sent via email to statistician for QC

AMGEN

CAST ACP Summary Tables - Examples

<Study/analysis specific Titles >

Table 1: Summary of the Assay Cut Point for SAV

Total Number of Observations	98
Total Number of Donors	49
Max	2.17
Min	0.61
Total Number of Observations Used in Analysis	97
Total Number of Donors Used in Analysis	49
Max	1.84
Min	0.61
SD	0.26
Back-transformed LS-Mean	1.02
ACP(95%) Upper	1.53
ACP(99%) Upper	1.82

Summary statistics of Max, Min and SD are calculated from raw data
Transformation Used in Analysis: Log Transformation
CAST-CI Execution Experimental Design # 2 Output
Data Source:

Table 2: Summary of Variance Component Analysis on SAV

	Variance	% Total
ANALYST		0 †
DATE(ANALYST)		0 †
PLATE(ANALYST*DATE)		0 †
DONOR_ID	7.54E-03	69%
Residual	3.45E-03	31%

Estimated Total Degrees of Freedom = 65.10

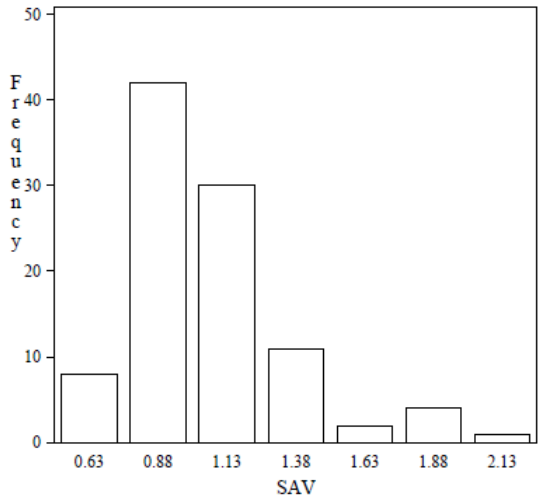
† Variance components set to zero when component is not significant.
CAST-CI Experimental Design # 2 Output
Data Source:

Table 3 is the listing (not shown)

AMGEN

CAST ACP Graphs - Examples

Figure 1: Distribution of SAV



Log-Transf.

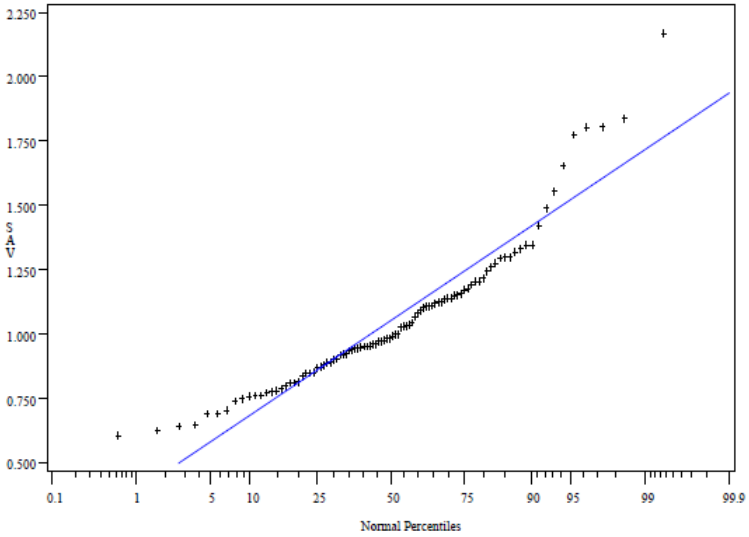
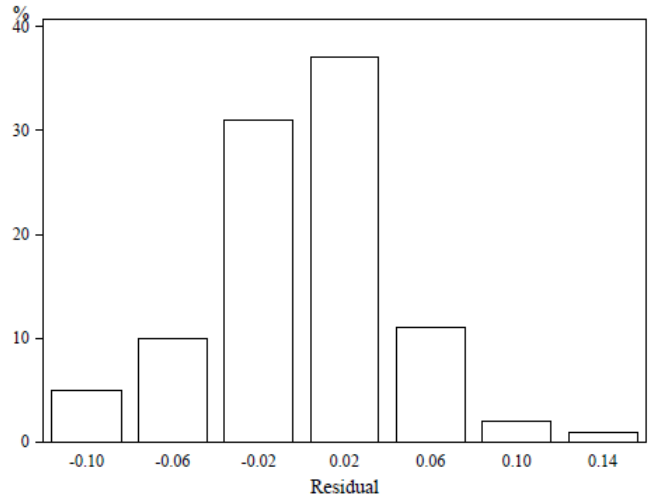
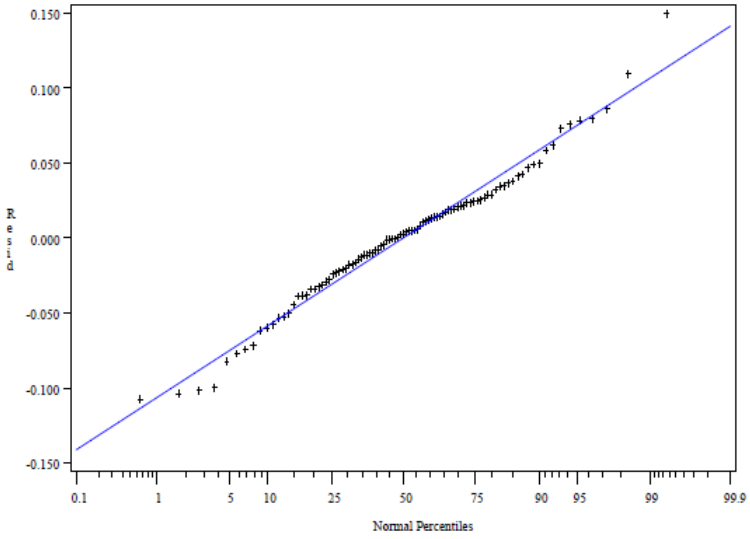
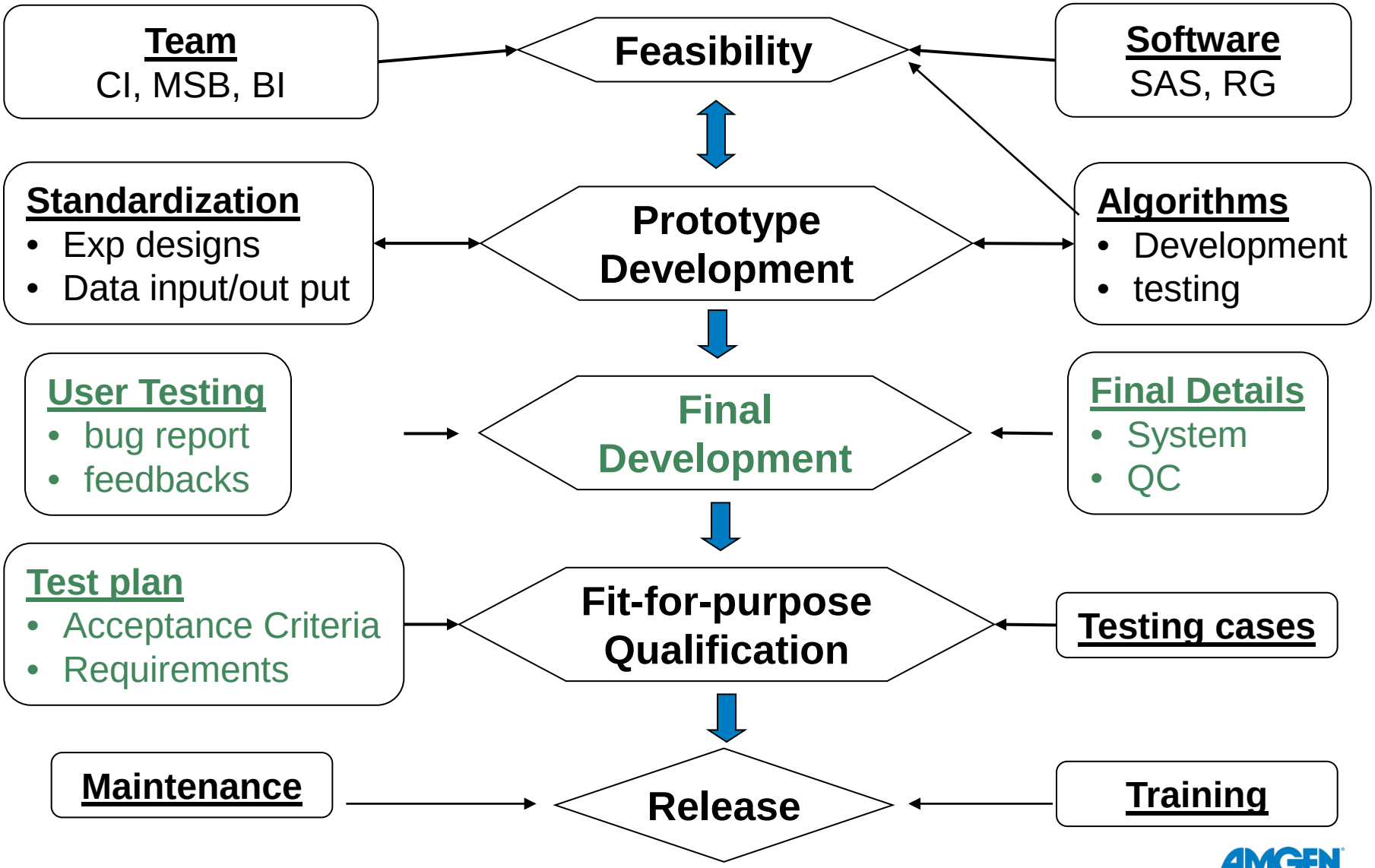


Figure 2: Distribution of Residuals (After Removing Outliers)

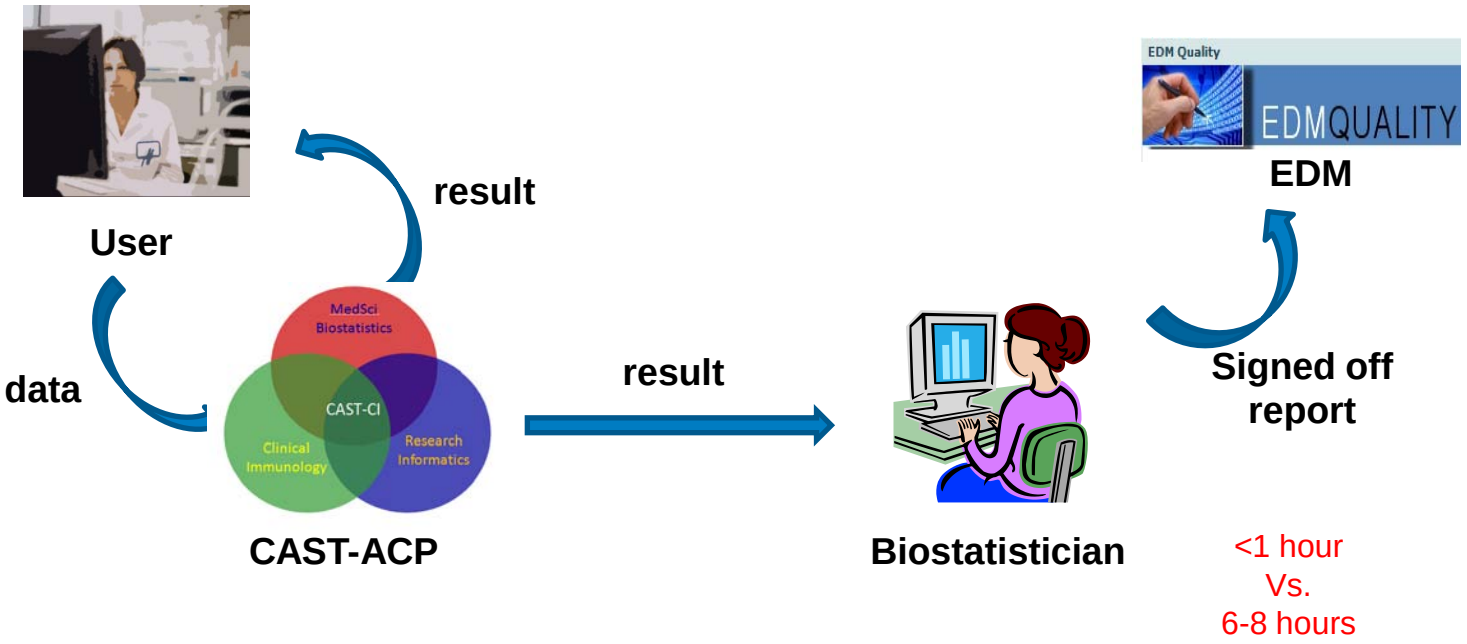




CAST Development Process



Standard Process



Benefit

- Used widely within Amgen
- Shorter turnaround and less time spent
- Encourages broader use of proper statistical analysis
- Improve compliance

Summary

- CAST ACP standardizes data entry, study design, automates assay cut point estimation, produces standard reports and facilitates review process
- This approach increases efficiency, maintains quality and improves compliance
- It also encourages proper study design and broader use of statistical analysis on assay cut point estimation
- Similar approach is being applied to other applications

CAST-ACP Project Team

R&TS Biostatistics

Cheng Su

Lei Zhou

Winnie Weng

Yuna Wu

Biometric Solution

Paul Hamilton

Mark Vanderstay

Research Informatics

Jayanthi Subramani

Clinical Immunology

Don Zhong

Naren Chirmule

Jenny Hu

Ami Bautista

Kortney Davis

Vineet Tadkod

Francesca Civoli