Experience in Clinical Immunogenicity Testing: Switching from Validation to Routine Analysis

Szilard Kamondi

Roche Pharma Research and Early Development, Pharmaceutical Sciences, Large Molecule Bioanalytical R&D, Roche Innovation Center Basel

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Outline

- Challenges
- Background information
- **CASE STUDIES**
- Conclusions

Challenges in Immunogenicity Testing

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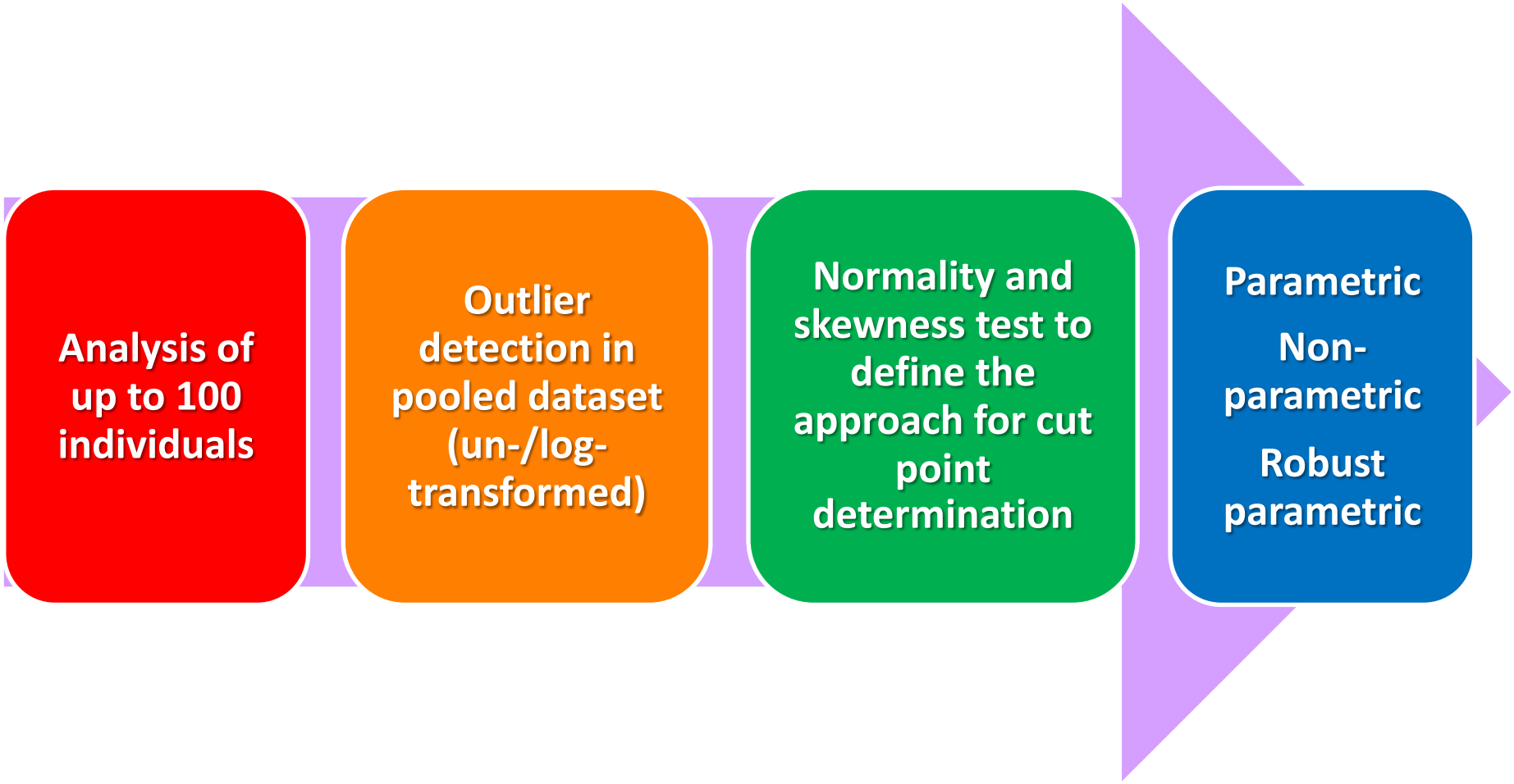
- Outlier detection / cut point determination
  - Complex statistics in combination with supersensitive methods
- LPC level selection (1% failure rate)
  - Based on limited number of experiments within a short period of time
  - 1 vs 2 LPC levels (sLPC and cLPC)
- Sensitivity / drug tolerance
  - Surrogate positive control
- Validation parameters
  - Stability? (BT, F/T, LTS)
- **Switching from validation to routine analysis**
  - **Commercially available pre-study validation samples vs. in-study population**
- Many more…

Data Evaluation Scheme



Positive Rates

Definitions

TPR

- Total Positive Rate: percent of all screened positive samples

FPR

- False Positive Rate: percent of screened but not confirmed positive samples

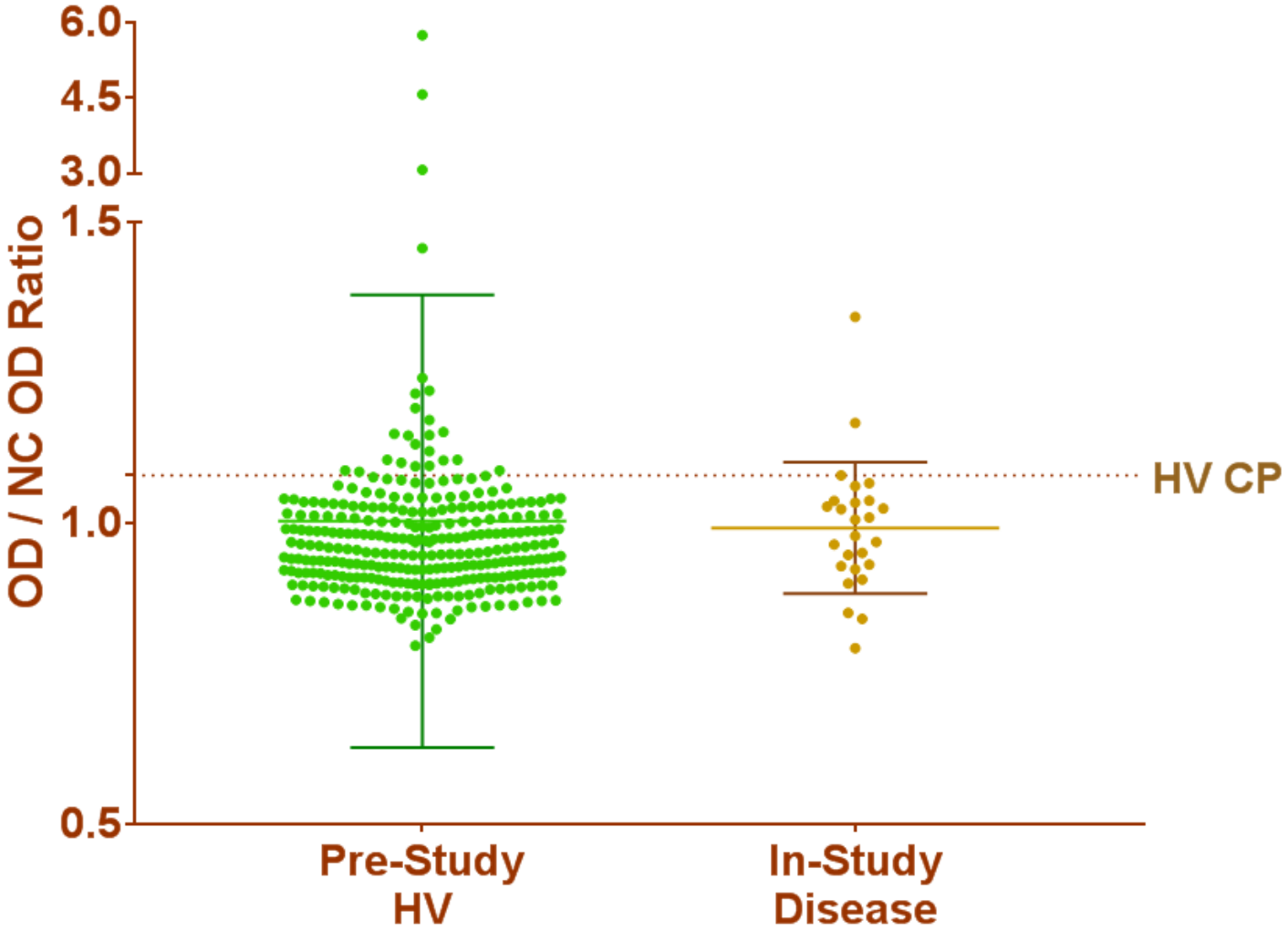
Important Aspects of Switching to In-Study Cut Point

- To avoid not detecting early immune responses
- To avoid detecting too many false positives
  - Target FPR in clinical screening assays: 5%
  - Acceptable range: 2-11%
- Pre-study validation with HV vs. commercially available disease samples
  - Latter may not bring any benefit

Case Study I

- Pediatric population
  - Minimized blood volume
- PEGylated-protein
- Bridging ELISA with a sensitivity<10 ng/mL
- Healthy volunteer (HV) validation data set with a few extreme outliers
- Screening cut point factor varies between 1.080 and 1.091 (pre-study TPR: 8.0 – 9.7%)
  - Robust parametric (outlier detection: no, 1.5*IQR, 3*IQR)
  - Parametric (1.5*IQR, 3*IQR, adjusted boxplot)
- Confirmatory cut point: ~10%

Case Study I


6.0
4.5
3.0
1.5
1.0
0.5
OD / NC OD Ratio
HV CP
Pre-Study HV
In-Study Disease

Case Study I

- Statistical test shows significant difference in variances
- In-study baseline TPR is 7.7% (2/26)
- No confirmed positive sample

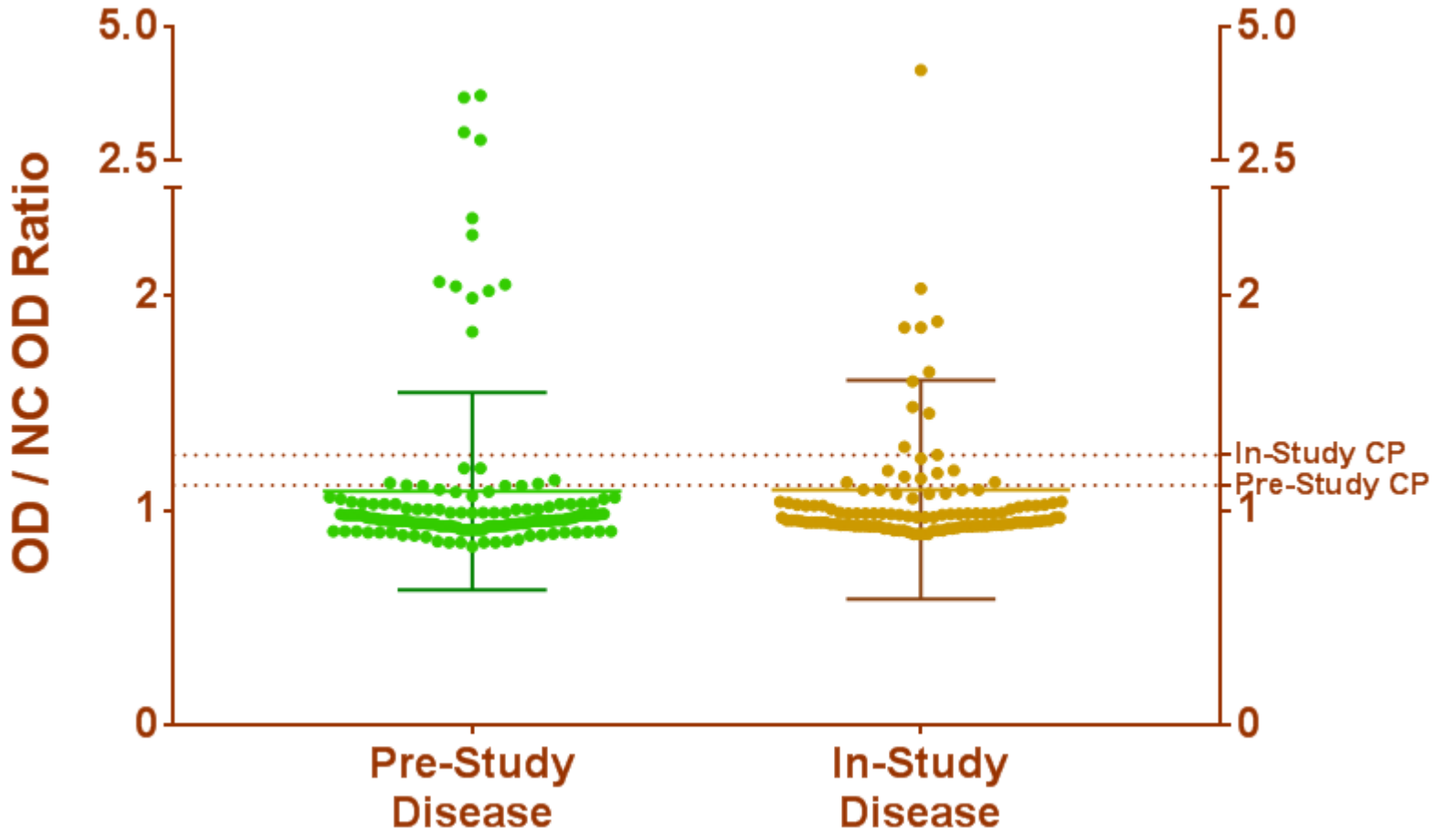
RE-ASSESSMENT OF CUT POINTS

AND

RE-EVALUATION OF SENSITIVITY / DRUG TOLERANCE

NOT REQUIRED

Case Study II



Case Study II

- Classical monoclonal antibody
- Bridging ELISA
- Visual inspection shows nice agreement between pre-study and in-study disease population
- Statistical test does not show significant difference

But…

- Robust parametric methods independently from the outlier detection methods (no oulier detection / 1.5*IQR / 3*IQR) result in similar but fairly high in-study TPR (23-26%)
- Using adjusted boxplot, the switch from pre-study CP (1.116) to in-study CP (1.277) reduces the in-study TPR from 17% to 9%
  - Benefit in large Phase III trials
  - Higher TPR might be tolerated in small trials

Case Study II

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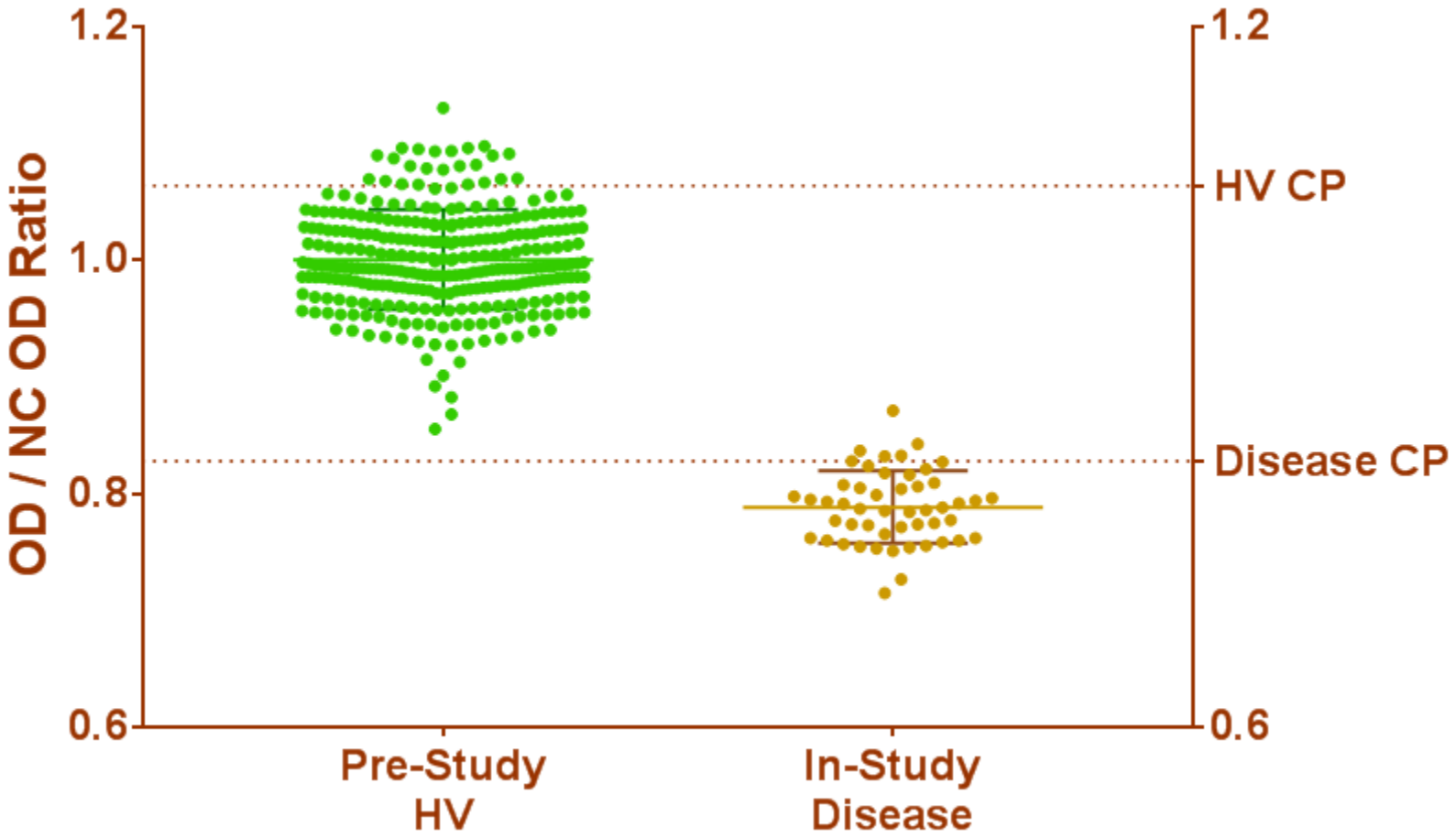
- At the same time negligible loss of sensitivity; 0.5 ng/mL -> 2.0 ng/mL
  - Sensitivity can be re-assessed using the pre-study validation curves
  - Intersection of the curve and the new/in-study cut point equivalent read-out
- LPC might be re-assessed to avoid increased plate failure rate due to LPC not meeting plate acceptance criteria
  - Re-assessment by using the pre-study validation curves
  - Study specific LPC levels may increase complexity in the lab
- No re-assessment of drug tolerance
  - Uncertainty in drug tolerance values is larger due to the surrogate positive control than such a small change in the CP value

Case Study III



Case Study III

- Obvious significant difference (statistically, viusally, FPR = 0% using pre-study CP) -> switch to in-study CP
- Challenges with plate acceptance control strategy and assessment of sensitivity
  - Limited volume of disease matrix
  - Due to the much lower read-out signals of the disease population, appropriate LPC cannot be prepared in HV NC pool
  - Existing LPC, prepared in HV NC pool, in combination with in-study CP is not appropriate to control method performance
    - Expected plate failure rate ~0%

Case Study III

- Solution
  - LPC (in HV NC pool) for plate acceptance using pre-study CP
  - Samples analyzed and categorized using in-study CP
  - Sensitivity is re-assessed by performing selectivity testing in disease individuals
    - In case of failure staggered increase of the LPC level
  - Drug tolerance can be re-assessed by using pooled disease samples if necessary and as volume allows

Case Study IV

- Immune system activator in oncology
- FPR = 0% in EiH baseline samples
  - Should we switch to in-study CP?
- Dose escalation is going smoothly without safety findings
- Unfortunately, immunogenicity incidence >50% after a few cycles
- Characterized immune response maturation (IgM -> IgG)
- Loss of exposure related to immune responses above a certain titer threshold

WELL CHARACTERIZED CASE

NO NEED TO SWITCH TO IN-STUDY CUT POINT

Conclusions

- Primary assessment by checking in-study screening positive rate in baseline samples
- Statistical tests and/or visual inspection of mean and standard deviation can be misleading
- LPC level might be adjusted, however, a target range for failure rate should be considered instead of using the strict 1% threshold
- Commercially available disease matrices might not bring any benefit
- Re-assessment of sensitivity by using the sensitivity curves from pre-study validation or performing selectivity testing with in-study baseline samples
- Rarely a black-and-white situation. Look at the big picture!

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… and many more

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Doing now what patients need next