LOW-LEVEL CHIMERISM DISCORDANCE BETWEEN STR AND NGS ASSAYS HIGHLIGHTS THE IMPORTANCE OF SENSITIVE DETECTION IN TRANSPLANT TOLERANCE

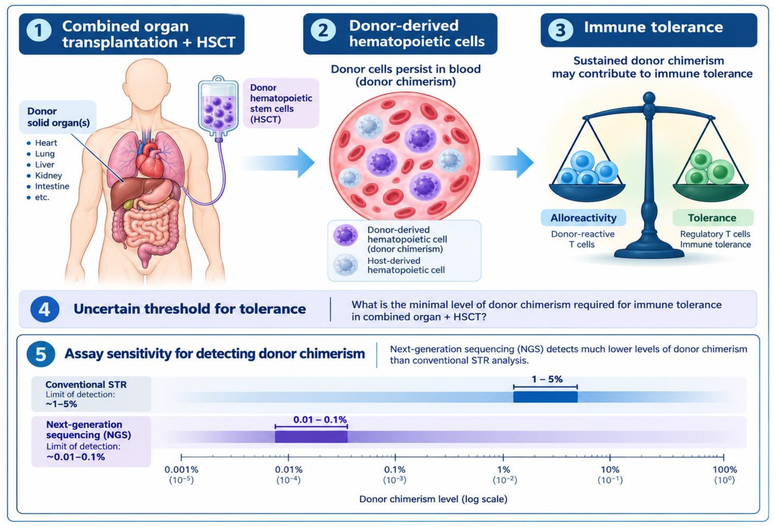
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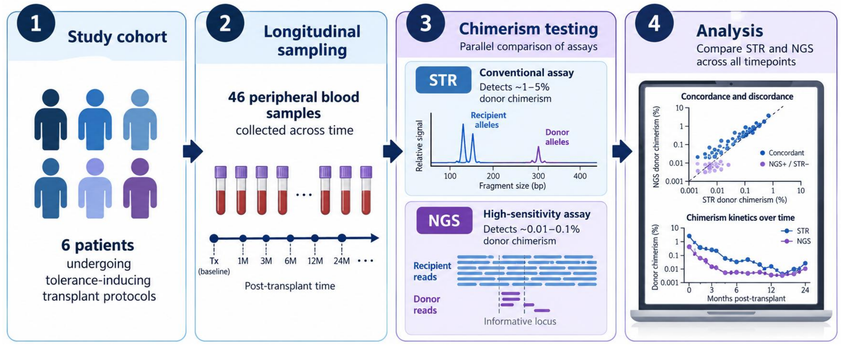
AIM

Sustained donor chimerism is believed to play a key role in immune tolerance following combined organ and hematopoietic stem cell transplantation (HSCT).

However, the minimal level of donor chimerism required for tolerance remains poorly defined, in part due to limited sensitivity of conventional short tandem repeat (STR) assays at low levels. Improved resolution in the low chimerism range may provide important insights into tolerance mechanisms.



METHODS

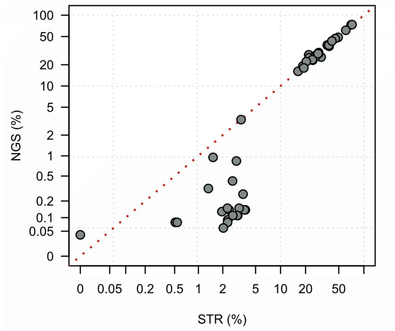


We evaluated longitudinal peripheral blood chimerism using both STR- and next-generation sequencing (NGS)-based assays in 46 longitudinal samples from six patients undergoing tolerance-inducing transplant protocols.

Concordance between platforms was assessed across the full range of donor chimerism, with particular attention to samples with low-level donor signal.

RESULTS

A



B

Patient	Days post-HSCT*	First STR <1%**	First NGS <1%***
PID1	1434	None	None
PID2	1003	659	380
PID3	1007	684	503
PID4	901	None	124
PID5	992	None	320
PID6	656	None	82

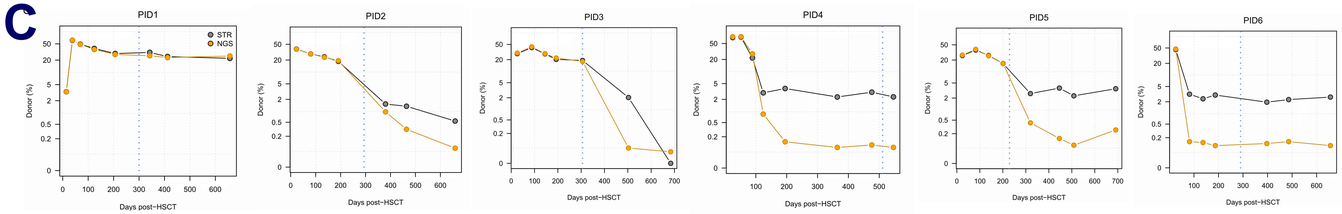
*Current days post-hematopoetic stem cell transplant (HSCT)

**Days post-HSCT of first STR result <1% chimerism

***Days post-HSCT of first NGS result <1% chimerism

'None' = no results <1%, 'PID" = anonymized patient identifier.

C



Concordance between STR and NGS assays was high in samples with moderate-to-high chimerism (Spearman's rho = 0.94, p < 0.001) (**Figure Panel A**).

However, discordance was greatest at low chimerism levels (<5%) (Spearman's rho = 0.44, p = 0.04), including NGS-only detection and quantitative differences affecting timing of decline to <1% (**Figure Panel B**). Low-level signals were observed in clinically stable patients.

Following tacrolimus withdrawal, NGS-detected chimerism declined to <1% over a variable timeframe (3 months - 1.5 years), with no consistent association with immunosuppression withdrawal (**Figure Panel C**).

This pattern was observed across HLA-matched/mismatched pairs and both simultaneous and delayed HSCT protocols.

SUMMARY/CONCLUSIONS

- Differences between STR and NGS-based chimerism assays are most pronounced at low chimerism levels, highlighting a limitation of conventional methods in tolerance monitoring. These findings suggest that the threshold of donor chimerism required for immune tolerance may be substantially lower than previously appreciated and may fall below the detection limits of STR-based assays.
- Together, these data support the use of higher-sensitivity NGS-based approaches to better characterize low-level chimerism and refine biologically relevant thresholds for tolerance. Future studies incorporating lineage-specific chimerism, particularly within CD3+ T cell compartments, will further define the role of microchimerism in tolerance induction and maintenance.

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