FAQ: ANC By Duffy Status

1. **What is the ANC by Duffy Status and its significance?**

Reference ranges are intended to identify the central 95% of healthy populations. However, some laboratory values can vary among different populations like von Willebrand factor by blood type. Another example is found the Duffy null phenotype and absolute neutrophil counts (ANC). Approximately two in three people in the United States who have African or Middle Eastern genetic ancestry have the Duffy-null phenotype. This results in a clinically insignificant lower ANC compared to the commonly used reference population which is often established from individuals of Asian or European descent of whom nearly 100% are Duffy non-null. Thus, individuals with the Duffy null phenotype have no increased risk of infection but are often incorrectly labeled as having neutropenia. This can result in unnecessary, expensive, and invasive testing, delayed or discontinued chemotherapy or other critical medications, exclusion from clinical trials, and other negative consequences.

2. **Are there costs or compensation for the Healthcare Systems?**

ASH has partnered with the Doris Duke Charitable Foundation and has funding available to help with the development and dissemination of Duffy-null specific reference ranges. This funding is intended to cover the costs of Duffy typing and ANC testing and patient recruitment as well as some support for the time from physicians, laboratory techs, and/or research assistants required to complete this project.

3. **Is this project assessing pediatric or adult populations?**

This project will attempt to validate adult reference ranges from previously published values which requires approximately 40-60 Duffy null samples. It will also attempt to establish new pediatric reference ranges which require 120 Duffy null samples per age category. Healthcare systems can opt to participate in adults only, pediatrics only, or both.

4. **How is data collected and managed in the study?**

There are multiple possible methods to obtain samples from healthy populations that are outlined in the draft protocols in order to match preferences and realities of each individual institution. Samples must be collected from healthy participants. However, residual blood or fresh blood can be used. Any setting where there is a density of healthy participants is acceptable. Duffy phenotyping or genotyping are both acceptable, and institutions can type samples in-house or through send-out. All institutions involved must participate in a central IRB. De-identified limited demographic data, Duffy typing, and ANC values will be submitted at regular intervals to RedCap Cloud throughout the project.

5. **How long will it take for the application to be approved?**

The anticipated timeline for application review, selection, and notification is approximately two months. The review process includes finalizing the Central IRB relationship.

6. **Can you give examples of how patients were identified and what methods you used?**

The original project focused on an adult-only population in a primary care setting, aiming to capture relatively healthy patients. Exclusions were based on chronic medical conditions and medications that could impact neutrophil count. Various settings like community centers, elective surgeries, or outpatient

clinics could be considered as sites to recruit healthy patients or volunteers. Duffy testing methods included phenotyping and genotyping. These can be done in- house or through send-out.

7. **Is it required that both pediatric and adult hematology are participating, or can it just be one group of hematology as well as someone from pathology?**

One group of hematology is sufficient. We want one patient-facing leader and a pathology leader. The patient-facing leader could include hospitalists or primary care doctors in addition to adult or pediatric hematology. The key is having both a patient-facing and laboratory-based representative for effective reference range development.

8. **Regarding leadership buy-in and support, is it hospital leadership, department chairs, or open to interpretation?**

We are flexible with leadership buy-in and support and it can include division heads of hematology, department heads of pediatrics or medicine, or any stakeholder supportive of implementing reference ranges at the institution. The focus is on obtaining support from individuals who can facilitate the success of the project.

9. **Do you have a data case report form? Are you collecting patient background, or just comparing two groups based on Duffy phenotype?**

We do have data case report forms (CRFs) that are shown here:

1. Age at time of ANC blood draw
2. Sex
3. Race (if available)
4. Ethnicity (if available)
5. White Blood Count
6. Absolute Neutrophil Count
7. Duffy status

10. **How will the identified data be shared, and what steps are being taken to ensure patient privacy?**

The identified data will be shared centrally with RedCap Cloud to facilitate the development of pools of data necessary for each age group, particularly for pediatric populations. Participating sites are requested to input data at least once every 2 weeks. A central IRB through Western Copernicus Group will be used. Privacy and confidentiality are paramount, and specific templates for protocols and consent forms are being finalized. The intention is to share and disseminate results widely while maintaining strict privacy safeguards.

11. **Did you only enroll subjects identified as black?**

Individual institutions can choose what is best for them. The original project only enrolled those identifying as Black as this population has the highest prevalence of the Duffy null phenotype. Institutions can be race or ethnicity agnostic, but this may mean that more samples are required to be collected to meet goals which may increase costs.

12. **Does the PI for pathology need to be a physician, or can it be someone else who works in the Blood Bank or hematology lab?**

There are no strict qualifications required. The most important aspect is to partner with someone who has the power to define reference ranges and publicize them in the electronic medical record. In the previous project, we worked with the hematology lab supervisor as well as the pathology director.

13. **How many patients are you expecting from each site?**

The number of patients expected depends on whether the site is doing adult reference range work, pediatric reference range work, or both. We require at least 20 Duffy null samples for adults. We ask for 200 pediatric Duffy null samples. However, there is some flexibility in the pediatric samples if this is a significant barrier.

14. **Can non-USA institutions apply?**

Due to Central IRB limitations, the focus is currently on U.S. institutions.

15. **Will you exclude patients with sickle cell anemia or those on hydroxyurea? Can you enroll patients with sickle cell trait? Are smokers excluded?**

Yes, patients on hydroxyurea will be excluded due to its impact on neutrophil count. Patients with sickle cell disease will also be excluded due to potential leukocytosis. Patients with sickle cell trait may participate, but we discourage a cohort that is >50% sickle cell trait. Smokers were not excluded in the original critieria, but this may be re-evaluated before study start.

16. **Can I enroll patients who are referred for neutropenia evaluation otherwise found to be healthy?**

No, these patients are not eligible for inclusion. They have already been noted to have ANC lower than the current reference ranges, and including these patients will inappropriately skew the normal reference range lower than what is an accurate reflection of the Duffy null population.

17. **Is there a preference for phenotyping or genotyping for transmissibility across centers?**

There's no preference. Both phenotyping and genotyping were done, and they showed close concordance. Institutions can choose based on availability and practicality.

18. **Who should be the lead for the grant application, hematologists, or pathologists?**

The lead should be a champion familiar with the project, and one of the PIs must be an ASH member. There is no preference if hematology or pathology is the lead applicant.

19. **Can abstracts be published on this information before the large collective data is published?**

While group publications are planned, individual abstracts may be acceptable with consultation with the oversight committee and PI.

20. **Can employees of a healthcare system be recruited as subjects for the DANC project during their annual physicals? Can incarcerated people be part of the study?**

Yes, it is acceptable from both a project and data collection perspective. From a coercion perspective, this is also acceptable as long as the same inclusion/exclusion criteria are used and the project obtains approval for this special population from their local IRB.

21. **Would Healthcare Systems be eligible to participate in this initiative if all patient labs, including CBCs and RBC Duffy antigen phenotyping, are sent to outside commercial labs like LabCorp or Quest, instead of being processed at the Healthcare System's institutional lab?**

We are not mandating a central lab for testing, so any CLIA-approved laboratory is sufficient and appropriately pragmatic for this project.

22. **Does the option for patients to have labs drawn at commercial laboratories impact the criteria for selecting the pathology principal investigator (PI)?**

The pathology PI is an important member of the team for purposes of buy-in and expertise for developing and instituting new duffy phenotype specific institutional reference ranges.