Male specific monoclonal antibody

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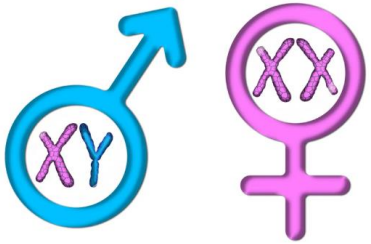
HEMOPHILIA A

- **Bleeding disorder** characterized by spontaneous bleedings at muscle and joint that result in disabling musculoskeletal damages and chronic arthropathy; replacement therapy is ineffective in 30% of patients

- **Affects** 24.6/100,000 male newborn and >**900,000 men worldwide***

- X-linked recessive disorder **caused by mutations** in a gene localized **on sexual chromosome X** coding for a clotting factor VIII

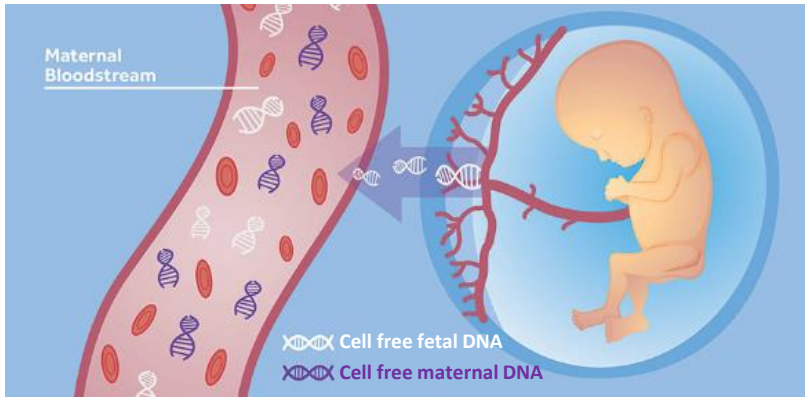
- **In males** (who have only one X chromosome), **one altered copy of the gene is sufficient to cause the disorder**, therefore males are affected much more frequently than females

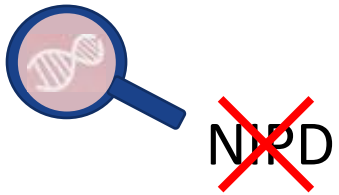


*Data from Annals of Internal Medicine 2019;171:540-546

PRENATAL DIAGNOSIS

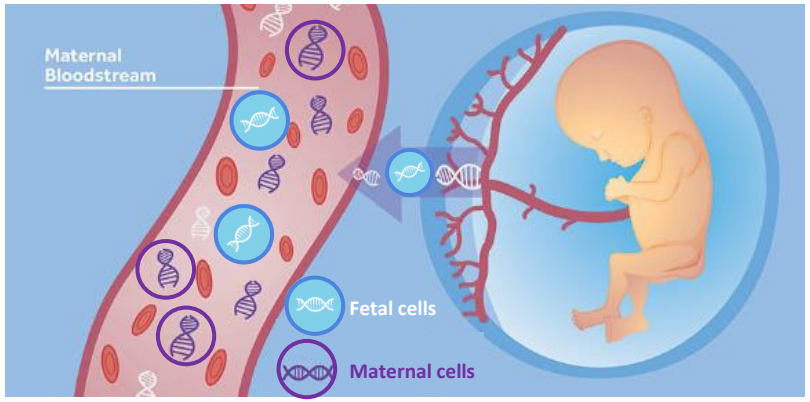
- **Useful to identify affected fetus**
- Based on **invasive diagnostic procedures** (chorionic villus sampling and amniocentesis) **with** an associated **risk of miscarriage**
- **Non invasive prenatal diagnosis (NIPD)**, based on analysis of circulating cell free fetal DNA (cffDNA), **is not feasible** in pregnant women carriers of X-linked recessive congenital disorder (such as Hemophilia A), since the poor maternally inherited portion of cffDNA is identical to the maternal one (>90%)

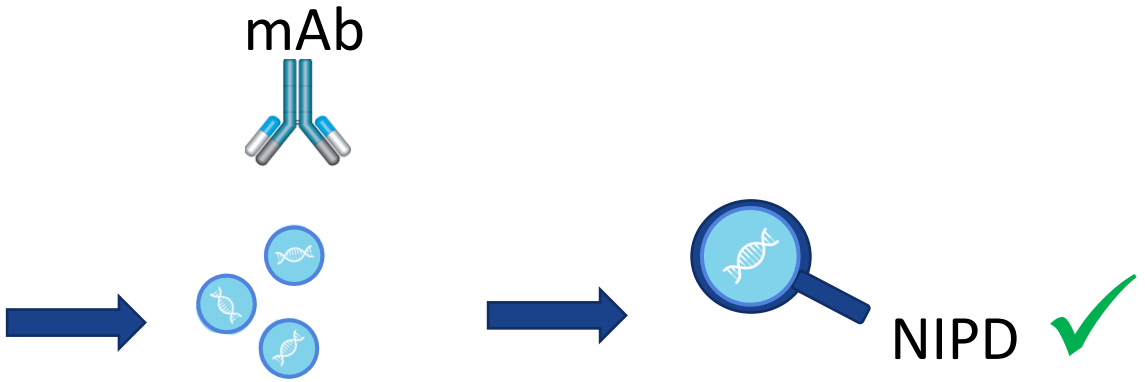




MALE CELLS SELECTION

- **Intact fetal cells** circulating in maternal blood **are ideal targets for NIPD** but the lack of validated, highly specific fetal biomarkers, which enable the unambiguous identification of fetal cells, has been the most limiting factor in all strategies up to now used for their isolation
- To identify male circulating fetal cells **we created a new antibody**, **selective for a male protein**, allowing their identification, useful for their isolation and for NIPD





IP STATUS

- The antibody and its use are protected in **two priority patent applications** (Italy and USA) filed in 2022

- A PCT patent application was filed in February 2023, claiming the Italian priority

VALIDATION

- **The specificity of the new antibody for male cells was confirmed in vitro on cell lines** (data published in “Development of a Specific Monoclonal Antibody to Detect Male Cells Expressing the RPS4Y1 Protein” Spena et al., Int. J. Mol. Sci. 2021, 22)

Cell lines

Biological samples

Diagnosis test

Clinical validation

VALIDATION

A

Cell lines

B

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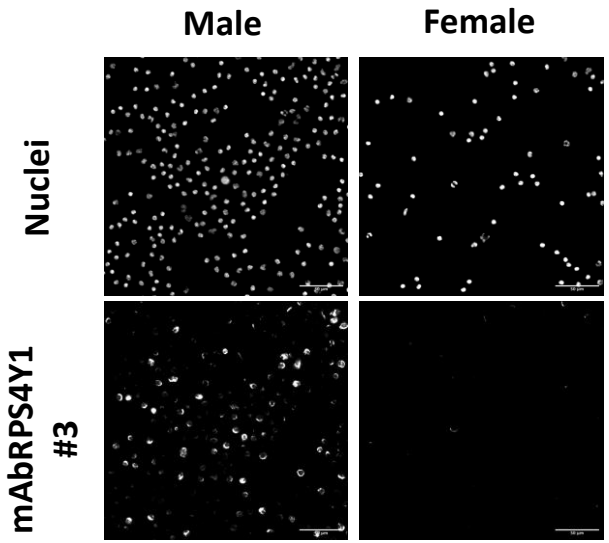
- Results have been confirmed on **peripheral blood mononuclear cells** and on **cord blood samples** (data unpublished)



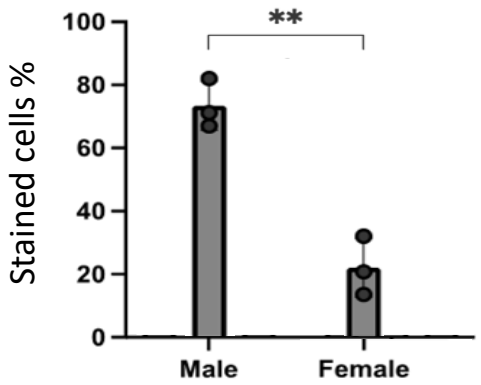


Figure: representative fields of view at 40X magnification showing nuclear (Male, Female upper panels) and mAbRPS4Y1#3 (Male, female lower panels) staining. (Male) cells from **umbilical cord blood** of a male newborn; (Female) cells from **umbilical cord blood** of a female newborn. Bar graph showing the results of the digital imaging analysis of mAbRPS4Y1#3 staining. The percentage of positively stained cells relative to total cells (y-axis) is reported for male and female samples (x-axis).

IMPLEMENTATION

- The antibody activity needs to be further validated in biological samples (blood and tissues)
- The antibody ability to identify male fetal cells circulating in maternal blood has to be validated in a diagnosis kit, testing its performance in laboratory and in clinic
- **The objective is the use of this antibody in a diagnostic kit and procedure for the reliable isolation of male fetal cells from the maternal blood and their future use for NIPD** of X-linked inherited diseases (such as Hemophilia A) and other disease in male fetuses

Cell lines

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TEAM

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