IAX Exchange

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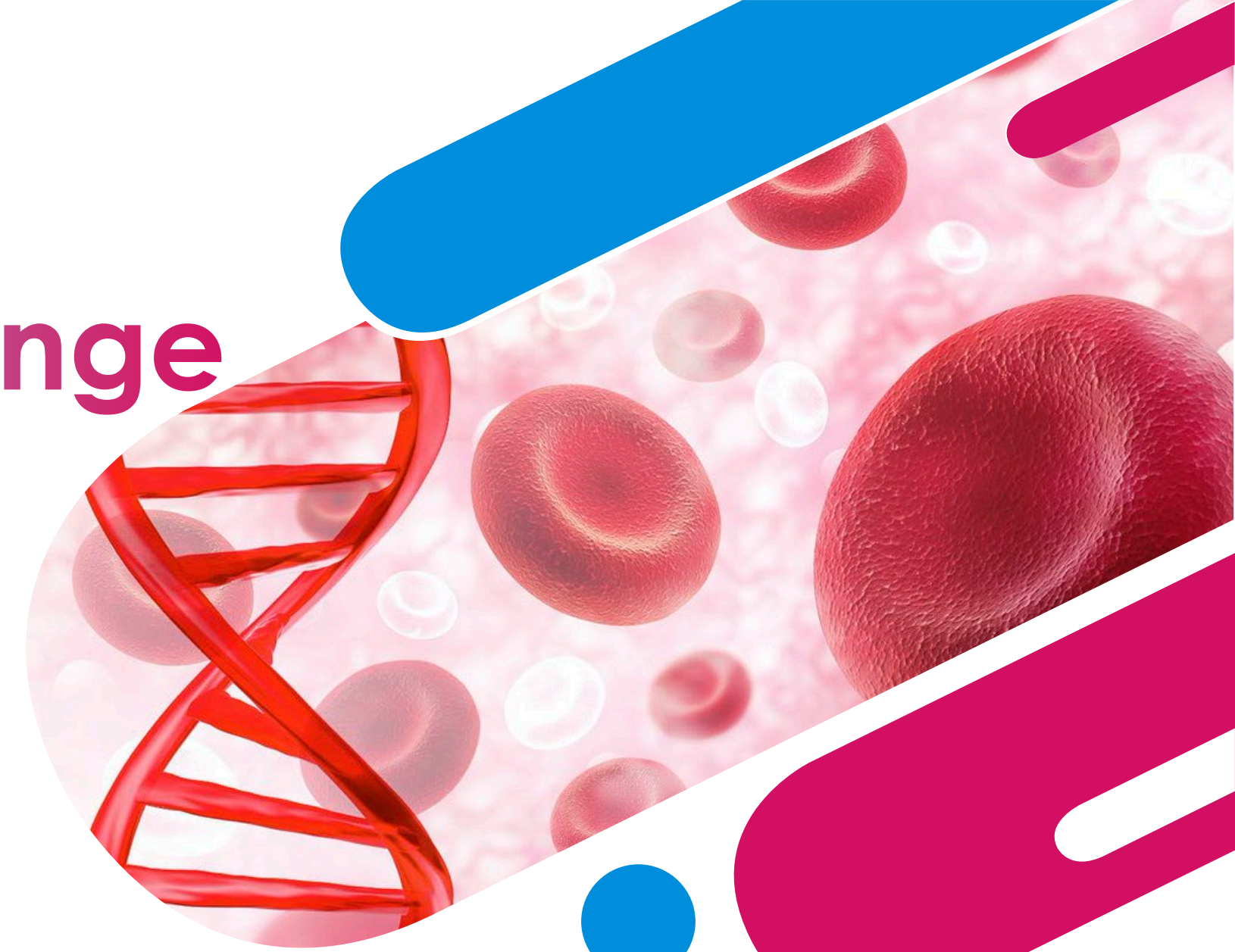


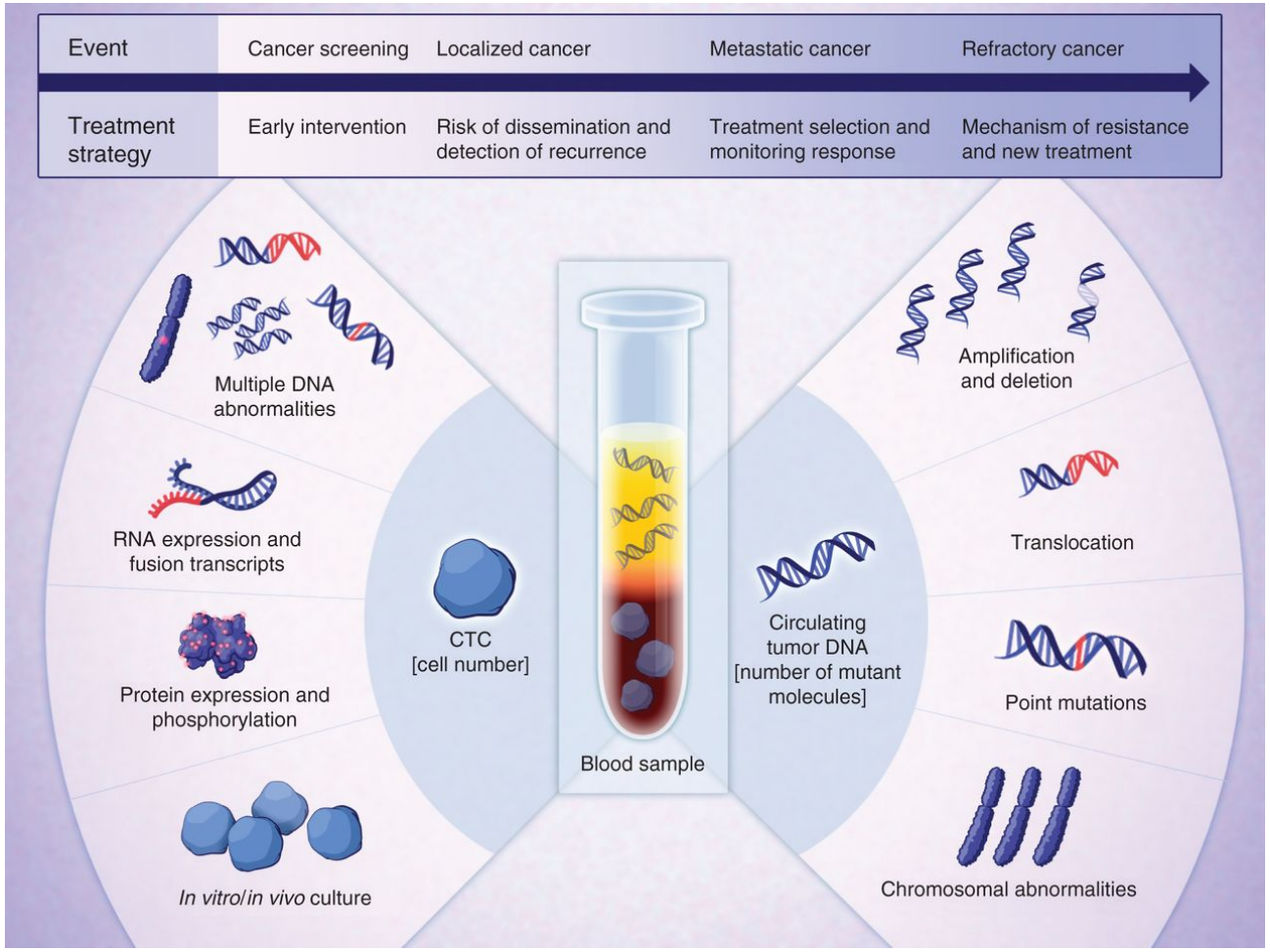
Aims of the project

- Learn methods and processes involved in Whole Exome Sequencing
- Help develop bioinformatics pipeline for easy and feasible variant detection for implementation
  - Knowledge exchange for accurate somatic variant detection
  - Implement on data derived from sequencing
- Develop custom panel based on Whole Exome Sequencing
- Build relationship for future collaborations

Endometrial Cancer

- Tends to have good prognosis
- Incidence is increasing
- 20% recurrence rate
- Recurrent EC is much more aggressive
    - Median survival < 1 year
- Treatment options are limited

Circulating tumour DNA



Patients & Samples

Four patients with EC; CT46, CT58, CT37 & CT14

Tumours were predominantly all high grade (3) except CT14 (grade 1)

- Matched tumour-normal samples were analysed
  - Tumour samples were formalin-fixed paraffin embedded
  - Normal samples derived from buffy coat containing lymphocytes

Patient	Histology	Stage	Grade
CT14	Endometrioid	1B	1
CT37	Endometrioid	1B	3
CT46	Serous	1A	3
CT58	Carcinosarcoma/Serous	4B	3

Methods

Cell3 Target Cell Free DNA Target Enrichment

Whole Exome Sequencing

- Library preparation and capture hybridisation protocol used as developed by Nonacus
- Initially trialled DNANexus – cloud platform with pipelines for variant calling
- Platypus, VarScan & ANNOVAR used
- Variant allele frequency calculated using:

$$\frac{Total\ number\ of\ variant\ reads}{Total\ number\ of\ reads} \text{ x } 100$$

Mutation Tracking

- Custom ctDNA panel developed based on results from Whole Exome Sequencing

Results

Whole Exome Sequencing

- High number of exonic nonsynonymous single nucleotide variants (SNVs) for CT14 & CT37 identified
- Germline variant analysis was conducted
  - Identify if patients had mutations in mismatch repair genes (Lynch Syndrome)

Patient	Number of exonic SNVs
CT46	59
CT58	52
CT14	301
CT37	131

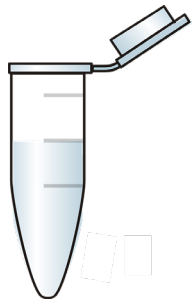
CT14 → Lynch Syndrome

CT37 → Lynch Syndrome

PMS2 p.G225C
PMS2 p.K435E
MLH1 p.I219V

PMS2 p.G751A
PMS2 p.K435E
PMS2 p.G59A
MLH1 p.I219V
EPCAM p.M115T

21 MUTATIONS IDENTIFIED WITH KNOWN IMPACT IN CANCER AND VAF > ~15%



Custom ctDNA panel

Outcome

Academia perspective

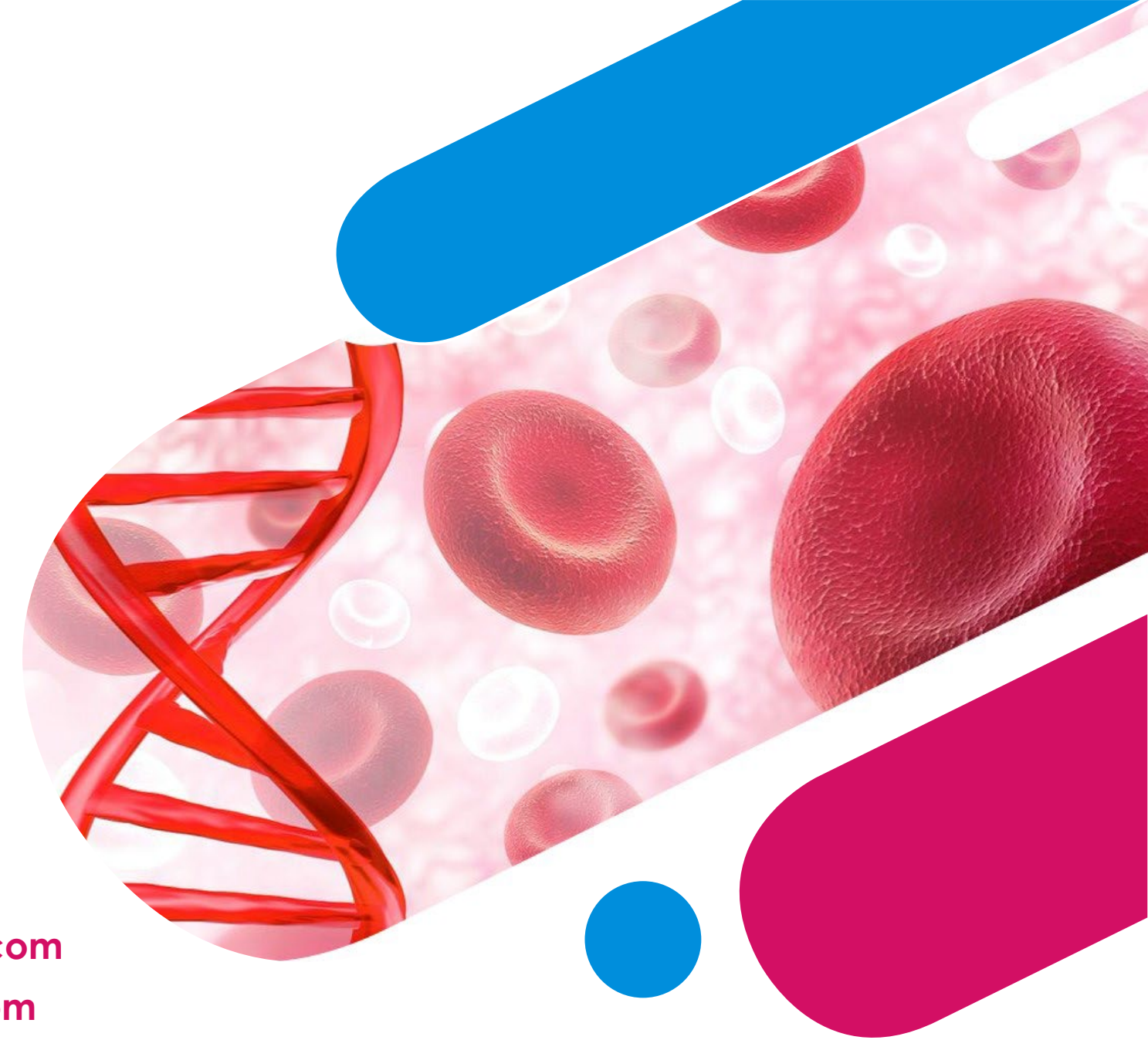
- Different working environment vs academia
- Skills needed to transfer to academia gained
- Better understanding of the science behind each process
- Highlighted benefit of working in collaboration with industry

Nonacus perspective

- Academic/clinical guidance on the benefits and use of our technology in this case endometrial cancer
- Access to clinical samples and validation of our technology across another cancer type
- We hope that further projects and development work and grant applications going forwards.

“This IAX grant has enabled clinical validation of our technology both in terms of determining the clinical need and validity of a test but also allows validation of our technology on real patient samples and for a given cancer type. ”

THANK YOU!



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