An overview of basic science discoveries that will impact clinical practice

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Expecting the unexpected.....

• Few scientific discoveries are predictable.
• In retrospect, many are obvious
  • At the time, not so.
  • The ‘black swans’
• This talk is about guessing where the next black swan will come from....
The funky stuff......

• Single Cell Technologies
• Gene Editing
• Manipulating Immunity
Single Cell Technology – Lab on a Chip
SPERMBOT
THE MICROFLUIDICS REVOLUTION

Today:
100ul
Single reaction tube

Vs

Tomorrow
1 picolitre
1000s of reactions per second
Lab-on-a-Chip Microfluidics

• Massive scaling up of capacity
• Reactions occur at picolitre volumes, thousands of times
• Greater sensitivity
• Fast – 1000s of experiments per second
  • Great for screening 10,000s of drug compounds – fast and less reagents
• Ability to study very rare cell populations
• Ability to carry out complex processes without massive lab facilities
  • PCR, qPCR, Cell sorting, Bacterial culture....
Applications

• Revolutionise PoC bedside diagnostics and monitoring
• Revolutionise single cell research
  • Clinical algorithms for patient stratification
• Massive opportunities for understanding:
  • HIV immunity,
  • drug discovery,
  • vaccine design
  • Latency and the reservoir
The funky stuff......

• Single Cell Technologies
• Gene Editing
• Manipulating Immunity
The Berlin Patient – over 5 years ago........
<table>
<thead>
<tr>
<th>Location of Transplantation</th>
<th>Age of Patient (yr)</th>
<th>Type of Cancer</th>
<th>Type of Graft</th>
<th>Outcome after Transplantation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Berlin†</td>
<td>40</td>
<td>Acute myeloid leukemia</td>
<td>HLA-matched unrelated</td>
<td>Alive after 7 yr, no viral rebound, no ART</td>
</tr>
<tr>
<td>Utrecht, the Netherlands‡</td>
<td>53</td>
<td>Myelodysplastic syndrome</td>
<td>Combined haploidentical bridge with umbilical-cord blood</td>
<td>Died from relapse of myelodysplastic syndrome and pneumonia after 2 mo</td>
</tr>
<tr>
<td>Münster, Germany§</td>
<td>51</td>
<td>Non-Hodgkin's lymphoma</td>
<td>HLA-mismatched unrelated</td>
<td>Died from infection after 4 mo</td>
</tr>
<tr>
<td>Essen, Germany¶</td>
<td>30</td>
<td>Non-Hodgkin's lymphoma</td>
<td>HLA-matched unrelated</td>
<td>Died from CXCR4-tropic HIV-1 rebound, died from relapse of non-Hodgkin's lymphoma after 12 mo</td>
</tr>
<tr>
<td>Minneapolis§</td>
<td>12</td>
<td>Acute lymphoblastic leukemia</td>
<td>Umbilical-cord blood</td>
<td>Died from GVHD after 3 mo</td>
</tr>
<tr>
<td>Santiago, Chile§</td>
<td>46</td>
<td>Non-Hodgkin's lymphoma</td>
<td>HLA-matched related</td>
<td>Died from pneumonia shortly afterward</td>
</tr>
<tr>
<td>Barcelona§</td>
<td>37</td>
<td>Non-Hodgkin's lymphoma</td>
<td>Combined haploidentical bridge with umbilical-cord blood</td>
<td>Died from relapse of non-Hodgkin's lymphoma after 3 mo</td>
</tr>
</tbody>
</table>
Can we inactivate HIV proviral DNA in latently infected cells?

4 gene editing techniques:

- **Cre recombinase**
  - Site-specific recombinase from bacteriophage enables precise genome editing by recombination between two DNA recognition sites (LoxP sites)

- **Zinc-finger nuclease**
  - Fusion proteins of nonspecific endonuclease cleavage domain of the FokI restriction enzyme with a custom-designed zinc-finger protein.

- **TALEN**
  - Transcription activator-like effector nuclease - from Xanthomonas TAL effector proteins

- **CRISPR-Cas9**
  - Most powerful gene-editing tool
“Clustered regulatory interspaced short palindromic repeat (CRISPR)-associated 9 (Cas9)”

• CRISPR loci and Cas proteins are present in ~90% of archaea and ~50% of bacteria

• Evolved as a defense against viruses

• A flexible and precise gene-editing tool. Two components
  • a short guide RNA (gRNA) is used to direct the sequence-specific cleavage of a specific target DNA.
  • and an endonuclease (Cas9) that cleaves both strands of the target DNA.

• Successful binding of Cas9 to the target and subsequent endonucleolytic cleavage causes a double-strand break (DSB).

• Repair is by ‘Non Homologous End Joining’ (NHEJ)
THE PRINCIPLE OF CRISPR/Cas9
How to apply gene-editing to the clinic?

- Ex vivo
- In vivo
How can we utilise gene editing for HIV?

- *Ex vivo versus in vivo*
- Only *ex vivo* trialed so far for HIV:
  - Zn Finger nucleases
How can we utilise gene editing for HIV?

• **Ex vivo versus in vivo**

• Only *ex vivo* trialed so far for HIV:
  • Zn Finger nucleases

• Recent data in humanised mice not discouraging

• Next step...human clinical trials??
CRISPR in Clinical Trials

  • PD-1 to be targeted in patients with metastatic non-small cell lung Ca.
  • Similar study recently approved by NIH and FDA.

• Conditions under consideration:
  • Malaria
  • Muscular dystrophy
  • Retinitis pigmentosa
  • HIV
  • ............and others.
The funky stuff......

• Single Cell Technologies
• Gene Editing
• Manipulating Immunity
Biologics – Can we improve on Nature?

• **Phase 1: HIV-1-specific mAbs (Now)**
  • Monoclonal, broadly neutralising antibodies:
  • VRCO1, 3BNC117, α4β7 integrin

• **Phase 2: Engineered antibodies (Tomorrow)**
  • Dual-affinity re-targeting (DART) proteins
  • Bispecific T cell engagers (BiTES)
  • Chimeric Antigen Receptor (CAR) T cells
  • Immune-mobilizing monoclonal T-cell receptors against viruses (ImmTav)
• 24 participants with chronic infection.
  • 3 & 8 doses, overlapping TI.
• All rebound after TI – after 4 or 5.6 weeks (mean)
• Significant delay at 4 weeks vs historic controls. Lost by 8 weeks
• Rebound virus showed evidence of resistant variants
HIV-1 antibody 3BNC117 suppresses viral rebound in humans during treatment interruption

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- N=13 with chronic HIV infection suppressed for >12 months
- Infusions of 3BNC117. TI 2 days later
- Up to 19 week delay in rebound vs historical controls (2.6 weeks)
- Rebound occurred with escape variants or once antibody levels had dropped
α4β7 integrin

- α4β7 integrin found on CD4 T cells & NK cells
- Mediates migration and retention of leucocytes in the gut.
- MAsCAM is natural ligand - constitutively expressed in the gut
- α4β7 integrin ‘high’ cells are preferentially infected by HIV
- Protection against mucosal transmission in SIV

- Question: Can monoclonals vs α4β7 integrin prevent viral rebound after TI?
Sustained virologic control in SIV\textsuperscript{+} macaques after antiretroviral and \(\alpha_4\beta_7\) antibody therapy

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Oct 2016, Science

• SIVmac239 infected macaques received ART and mAb vs \(\alpha_4\beta_7\) integrin.

Post TI:
• CD4 T cell restoration in blood, gut and peripheral tissues
• Two never rebound, 6 blip but then control.
  • Now between 1-2 years without rebound.
• Mechanism unclear - ? related to IgG vs Gp120 V2
• NEXT: chronically-infected humans: N=15; Phase 1 trial with Vedolizumab then ATI; already recruiting.
Fig. 1 Control of plasma and GIT viral loads.

Fig. 3 Immuno-PET–CT analysis confirms the preservation of CD4+ cells.
Vedolizumab to Cure HIV

- Effective therapy in IBD
- Stops cells homing to the gut
- Induces SIV remission in macaques...
- Takeda working with NIH to conduct clinical trials in HIV in humans
- Mechanism unclear
  - Will know more later this year......
Phase 2: DARTs and BITEs

- ‘Dual-affinity re-targeting’ (DART) molecules
- ‘Bispecific T cell engagers’ (BiTEs)

- Provide additional cytotoxic functions (the kill) to the immune responses
- Blinatumomab (CD19xCD3 BiTE) was recently approved for the treatment of acute lymphoblastic leukemia
DARTS

• Dual-Affinity Re-Targeting (DART) molecules
  • Bind CD3 – pulling in T cells
  • Bind antigen
    • eg anti-Env IgG antibodies

• Increase target killing
• Improve on natural immunity
• Phase 1 trials in AML, lymphoma and colorectal cancer
CD32a
the holy grail of HIV infection???
March 2017

“Marker 1” revealed:

CD32a – another new target......
CD32a is a marker of a CD4 T-cell HIV reservoir harbouring replication-competent proviruses

Benjamin Descours\textsuperscript{1,*}, Gaël Petitjean\textsuperscript{1,*}, José-Luis López–Zaragoza\textsuperscript{2,3,4}, Timothée Bruel\textsuperscript{2,5}, Raoul Raffel\textsuperscript{1}, Christina Psomas\textsuperscript{6}, Jacques Reynes\textsuperscript{6}, Christine Lacabarat\textsuperscript{2,3,4}, Yves Levy\textsuperscript{2,3,4}, Olivier Schwartz\textsuperscript{2,5}, Jean Daniel Lelievre\textsuperscript{2,3,4} & Monsef Benkirane\textsuperscript{1}

The persistence of the HIV reservoir in infected individuals is a major obstacle to the development of a cure for HIV\textsuperscript{1–3}. Here, using an \textit{in vitro} model of HIV-infected quiescent CD4 T cells, we reveal a gene expression signature of 103 upregulated genes that are specific enriched in inducible replication-competent proviruses and can be predominant in some participants. Our discovery that CD32a\textsuperscript{+} lymphocytes represent the elusive HIV-1 reservoir may lead to insights that will facilitate the specific targeting and elimination

March 15, 2017; Nature
CD32a identifies the CD4 T-cell HIV reservoir
If CD32a is a marker for the reservoir.....

- A biomarker for latent infection
- An opportunity to understand latency much better.
- A new lab test to assess cure-based interventions
- .....or even a new therapy: “anti-CD32a monoclonal antibody therapy”

“HIVCURIMAB”
Closing remarks

• The field of HIV has a track record translating basic science into clinical practice
• ART changes the questions faced by scientists and clinicians
• Work in HIV translates across to other fields (e.g. cancer) – and vice versa
• Time for a drink – look out for the black swans!

THANK YOU