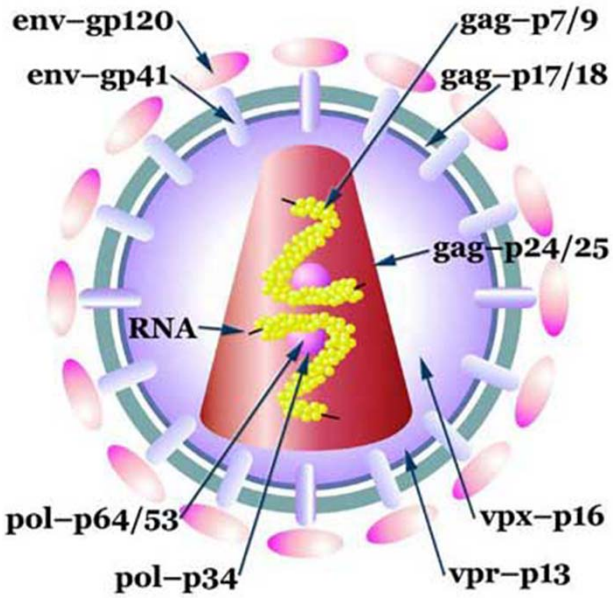
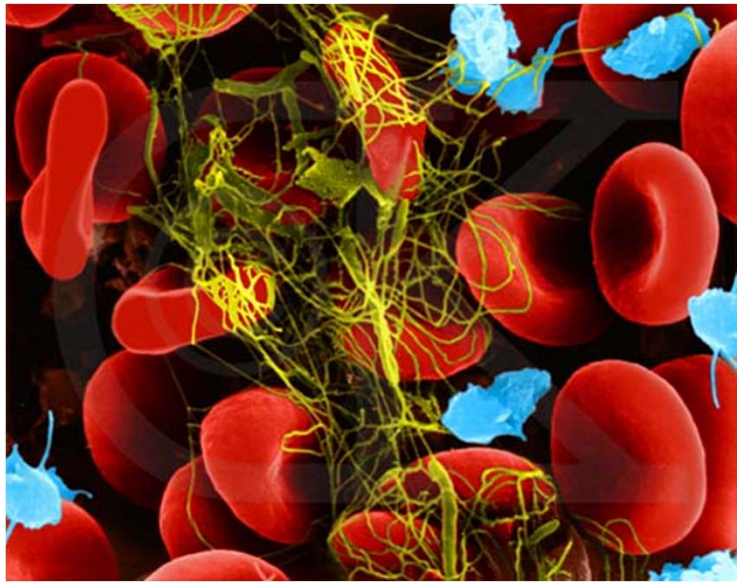


21-1-2018

Beyond ART:
HIV and Haematology

Htun Lwin Nyein

2018



Outline

- HIV and Cytopenia
- HIV and Coagulopathy
- HIV and Lymphadenopathy
- HIV and Immunotherapy

The haematological features of HIV infection

- Infection by the HIV and the consequent fully developed AIDS can have profound haematological effects in
  - the primary infection period
  - the phase of clinical latency, and
  - patients with advanced disease

HIV and Cytopenia: Prevalence

Cytopenia	Asymptomatic HIV (%)	Advanced HIV (%)
Anemia	10-20	70-80
leucopenia	20	85
Neutropenia	0-10	20-60
Lymphopenia	10	65-80
Thrombocytopenia	5-20	25-50

Etiology of Cytopenia in HIV-Multiple factors Involved

- Haematopoietic stem cell – not infected
- More committed myeloid progenitor cells:
  - may be infected
  - functionally abnormal
  - exhibit marked decreased colony growth
- Altered bone marrow microenvironment

HIV and CBC Study in Myanmar

Study parameters	Rai Mra (1993)	Hutn Lwin Nyein (2001)
Total patients	63	147
M:F	3:1	15:1
Mean age	30 yrs	25 yrs
Anemia (Hb≤ 10 g/dl))	60 %	42 %
Normocytic	60%	65%
Leukopenia (< $4x10^9/L$)	25%	20%
Neutropenia (< $2x10^9/L$)	22%	14%
Lymphopenia (< $1.5x10^9/L$)	70%	60%
Monocytopenia (< $2x10^9/L$)	22%	29%
Thrombocytopenia (<$150x10^9/L$)	28%	22%
Pancytopenia	8%	18%

HIV and Anemia

Anemia :

- is most common hematological abnormality.
- Is **an expression of active immune activation.**
- Is associated with disease progression and decreased survival.

Causes and Mechanisms of Anemia in HIV

Causes of Anemia	Mechanisms
↓ Red Cell Production **(↓retic count, Normal/↓ ID bilirubin)**	**A. Normocytic** - bone marrow infiltration(NHL, KS) - infection(MAC,TB,CMV,B19,Fungal) -HIV **B. Microcytic** : IDA (chronic blood loss) **C. Macrocytic**: Drugs(AZT, Chemo,RBV)
Ineffective production **(↓retic count, ↑ID bilirubin)**	Folate/ B12 deficiencies
↑ Red Cell Destruction **(↑retic count, ↑ ID bilirubin)**	+ AIHA + hemophagocytic syndrome + TTP + DIC + oxidative drugs: Dapsone, Sulpha

Treatment Options of Anemia in HIV

- **Correct the underlying causes:**
  - treatment of OIs
  - stop implicated drugs
  - hematinics replacement in deficiencies
- **ART**
- **Blood transfusion**
- **rHuEPO therapy**

HIV and Leucopenia

- Lymphopenia and Neutropenia commonly.
- Impaired granulopoiesis.
- Neutrophil function abnormalities.
- Autoimmune destruction.
- Peripheral blood film:

  Hypopigmentation, Shift to the left, peudo-pelgar huet and other dysplastic changes.

HIV and Neutropenia

Aetiology of Neutropenia

- Disseminated fungi may infiltrate bone marrow.
- Lymphomas produce pancytopenia through diffuse bone marrow involvement.
- Cytomegalovirus infection directly infects marrow stromal elements and myeloid cells.
- Anti neutrophil antibodies detected in 1/3rd
- HIV itself is a mediator of abnormal hematopoiesis in all cell lines.
- Direct infection of hematopoietic precursors
- Aberrations of local cytokine and growth factor signaling,
- Changes in the bone marrow stroma.
- ↓ (G-CSF)

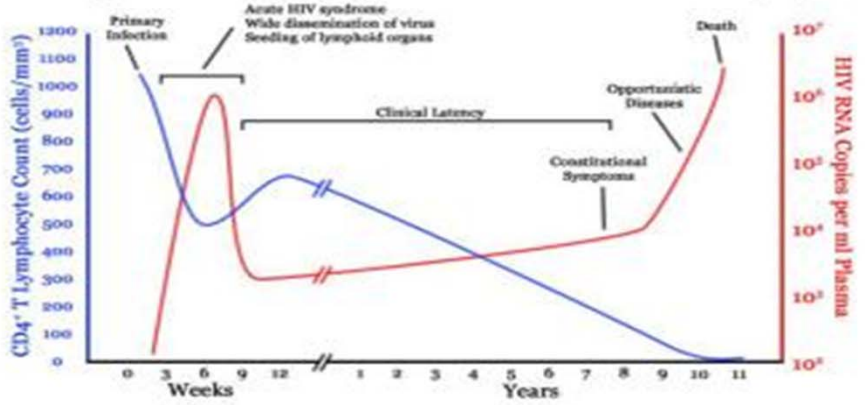
HIV and Neutropenia

- **Mechanism:**
  - : ↓ colony growth CFU-GM
  - : soluable inhibitory substance
  - : ↓ G-CSF level
  - : medications- AZT, TMP-SMX, Ganciclovir
- Risk of infection increased at ANC < $1x10^9/l$
- **Treatment** : stop implicated drugs, aggressive treatment of infection and use of G-CSF

HIV and Thrombocytopenia

THROMBOCYTOPENIA

- Common – 40% at some time
- May occur at any period of infection
- Worse with progressive immunosuppression
- Two groups:

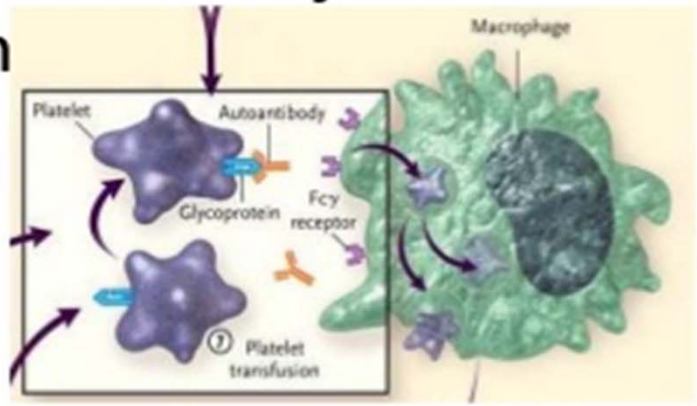


  - primary HIV-associated thrombocytopenia
  - secondary thrombocytopenia

HIV and Thrombocytopenia

Pathogenesis of thrombocytopenia

- Macrophages in the RES major mediators of platelet destruction



- HIV transcripts directly infect megakaryocytes
- ↓ in platelet production.
- ↑ apoptosis of megakaryocytes
- A spontaneous remission rate of almost 20 % in patients with PHAT.

HIV and Thrombocytopenia

- **Primary HIV associated ITP**: autoimmunity(cross reactivity between HIV gp160/120 and PLT gp IIb/IIIa)
- May occur early and at any time during course
- Generally correlates with degree of immunosuppression
- **Risk** increases with ↓CD4 count, untreated VIV, age>50 yrs, IDU, black race, anemia and with HCV co-infection.
- **Treatment**: ART(AZT), Steroid ± rituximab, IVIG, Anti-Rh Ab,
  : TPO receptor agonists
  : stop implicated drugs
  : Splenectomy

Causes of Bone Marrow Suppression in HIV

Infections/Tumours	Medications
HIV infection Mycobacterium infection Fungal infection Parvovirus B19 infection Lymphoma Myeloma Secondary metastasis	ART: AZT ddI, d4T Anti-viral: Ganciclovir, Forscanat Anti-fungal : Flucytosine, Amphotericin Anti-PCP: TMX-SMX, Pyrimethamine Anti-neoplastic: Chemotherapy Immune modifier: Interferon

HIV and Coagulopathy

- Thrombotic Thrombocytopenic Purpura(TTP)
- Thrombosis
- Antiphospholipid syndrome
- Acquired protein S deficiency

Thrombotic thrombocytopenic purpura (TTP)

- Big five of TTP
  - Red cell fragmentation
  - Thrombocytopenia
  - Fluctuating neurological disturbances
  - Renal failure
  - Fever

HIV and TTP

- Is a very serious but rare complication.

Pantad	Laboratory
Fever	Progressive anemia
Neurological	Progressive thrombocytopenia
Renal failure	Blood film: hemolytic picture, schizocytes
Hemolytic anemia	Evidence of hemolysis(↑LDH/ Retic/ID bilirubin)
Thrombocytopenia	↑creatinine
	Normal coagulation parameters

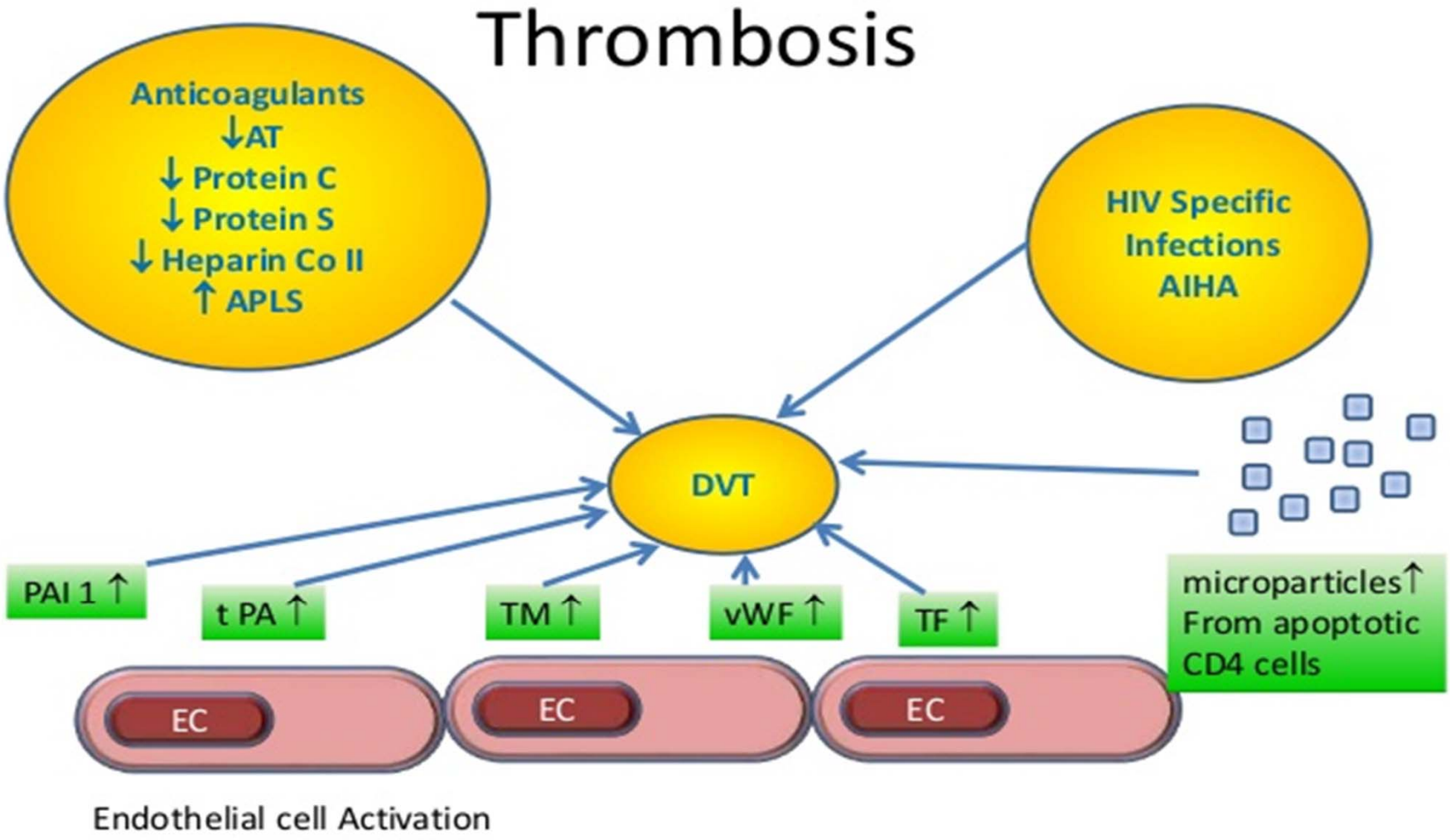
- Treatment: Plasma exchange, Steroid, ART, Rituximab

HIV and Thrombosis

Risk factors:

- Age > 45 yrs old
- Ois, CMV retinitis
- Malignancies
- AIHA
- Hospital immobility
- Use of megestrol/ estrogen
- APS, acquired protein S def, ↑Factor VIII/Fibrinogen
- Hyperlipidemia

HIV and Thrombosis



HIV and CBC

- **CBC finding suggestive of HIV infection** are:

  Unexplained anemia,

  leucopenia/lymphopenia, ± **eosinophilia,**

  thrombocytopenia singly or in combination.

- Thrombocytopenia may occur early and **is sometime the first manifestation.**
- Anemia and neutropenia/lymphopenia develop later, and with progression of disease.
- The incidence of the various cytopenia correlates directly with the **degree of immunosuppression.**

HIV and Lymphadenopathy: Causes

- Acute seroconversion
- PGL
- Mycobacterium tubertulosis
- Lymphoma
- Fungal infection
- Mycobacterium avium complex disease
- Kaposi’s sarcoma

HIV and Malignancies

- HIV infection is a well-established risk factor for tumour.
- Results in extraordinary increased risk of malignancies.
- **AIDS-defining tumour**:

  Kaposi's sarcoma – RR > 3000

  HG Lymphoma – RR > 100-300

  Cervical/Anogenital Tumour- RR 20-30

- **Non-AIDS associated malignancies**:

  Hodgkin's Lymphoma, Myeloma, Acute leukemia

  Testicular/ breast/ prostate/ lung and liver cancers

HIV associated Lymphoma (HAL)

- First reported in 1984.
- Aggressive B cell lymphoma classified as AIDS-defining Illness in 1985.
- Related to polyclonal B cell activation.
- **Types:**
- High Grade DLBCL Immunoblastic

  2 variants- Primary effusion Lymphoma (Body cavity lymphoma)

  - Plasmacytic lymphoma of oral cavity
- Burkitt' type lymphoma (Small non-cleaved cell lymphoma)
- Primary CNS lymphoma
- T-cell Lymphoma

HIV Associated Lymphoma : Pathogenesis

The heterogeneity reflects the various pathogenic mechanisms in lymphomagenesis

- Chronic B cell activation
- Genetic aberration, cytokine deregulation

- Polyclonal B cell expansion

- Acquired genetic mutation
- e. g. c- MYC oncogene in Burkitt Lymphoma
- BCL-6 mutation in DLBCL

HIV associated Lymphoma(HAL)

- Usually present with advanced stage of disease.
- More aggressive and more extensive “B” symptoms.
- Frequently extra-nodal (GIT, CNS, BM, Liver)
- Involved unusual sites (anus, Heart, body cavity, Jaw, gingival, soft tissues, muscle, rectum)
- Predominant associated with EBV and HHV-8.
- Less response to chemotherapy and high relapse rate.

HAL study in Myanmar

HAL	Htun Lwin Nyein (2004)	Aye Aye Gyi (2009)
Total	6	12
Age (mostly involved)	23-49 yrs	25-56 yrs
Male: Female	2.2:1	5:1
Stage III/IV	50%	75%
CD4 count <200	67%	78%
B cell NHL	83%	92%
High grade NHL	67%	75%

Characteristics Related to NHL at Presentation

SKIN INFILTRATION

LIVER

- ***Extra-nodal involvement*** : 75%
- ***Both nodal & extra-nodal (67%)***
- ***Isolated extra-nodal disease***: 3 cases (25%) oral cavity, rectum and ovaries
  - Common extra-nodal sites:
    - bone marrow (42%)
    - liver (25%)
    - skin (25%)

Aggressive, bulky disease: 42%

Advanced -stage III & IV: in 2/3

Treatment of HAL

- Pre-HAART era : poor outcome, median survival- 6 months
- HAART era: ART, OI prophylaxis

  : Chemotherapy – R-CHOP (CR 58%)

  R-DA-EPOCH (CR 73%)
- Caution in use of Rituximab at CD4 count <50.
- CNS prophylaxis may be required.
- Relapse/refractory NHL – HDT followed by PBSCT option.

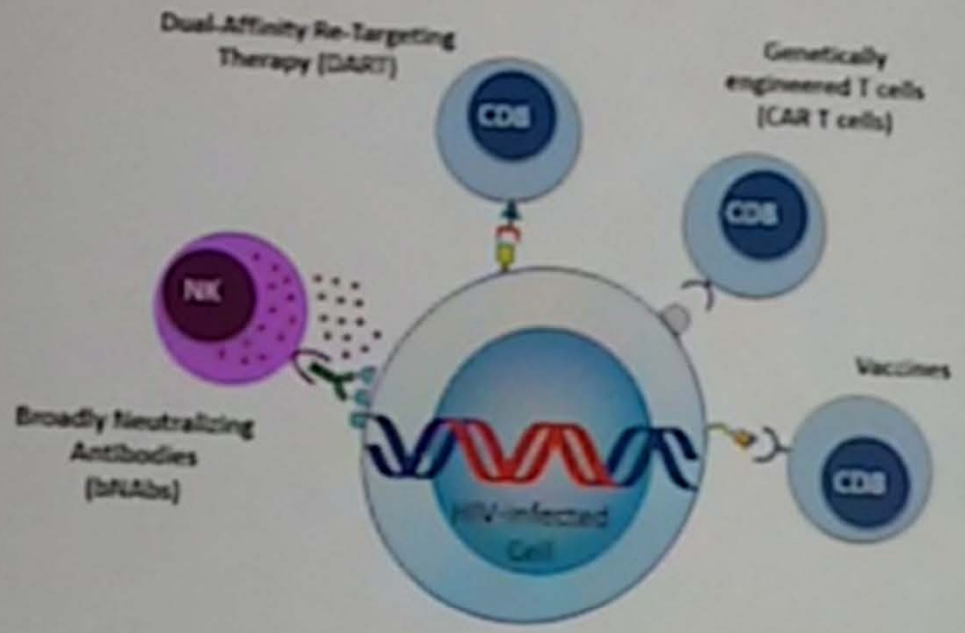
Therapy of HIV Associated NHL

- **Multifaceted challenge** :
  + Underlying immunodeficiency
  + Peripheral blood cytopenia
  + Involvement of CNS and bone marrow
  + Increased or concurrent risk of opportunistic infections
  + Overlapping severe haematological toxicities of therapy
  + Poor tolerability

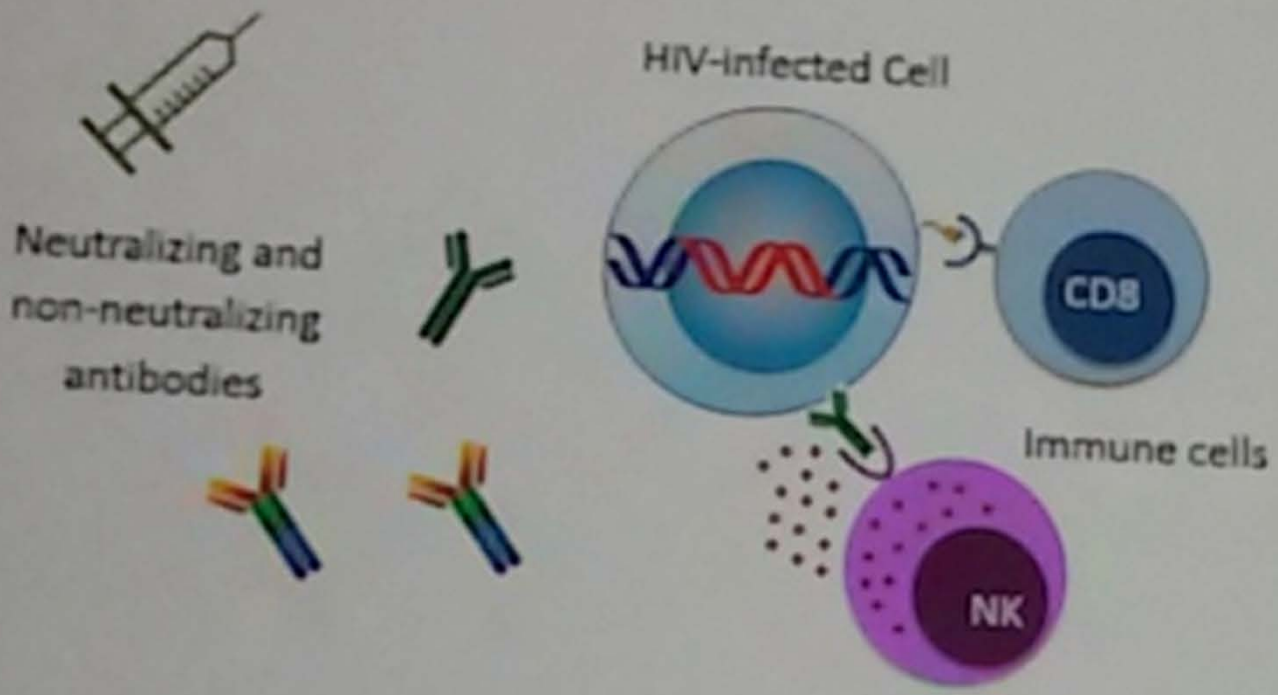
HIV and Immunotherapy

- Therapeutic vaccine
- Broadly neutralizing antibody
- PD-1 blockage therapy
- CAR- T cell therapy
- Gene therapy

Killing HIV-infected Cells



HIV Vaccines To Boost Immune Function



HIV vaccine studies in early treated adults in Thailand

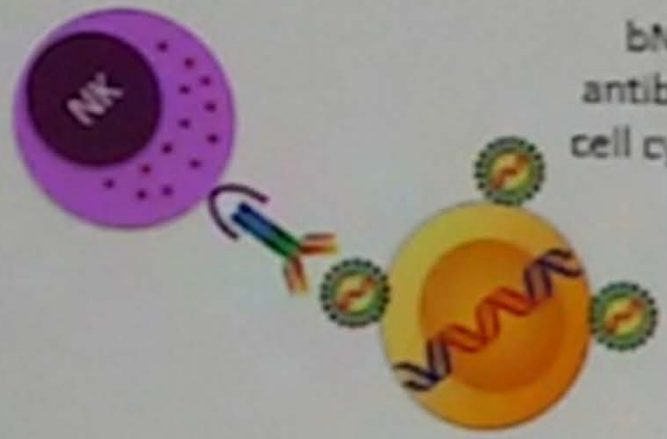
- Ad26/MVA
- Dendritic cell immunotherapy

Reiks-Ngarm, NEJM 2009; Haynes, NEJM 2012; Macatangay, JID 2016; Barouchi, Nature 2016; Bradley, Nat Commun 201

Results of Therapeutic Vaccine Trial

- Therapeutic vaccination in a group of HIV-infected individuals treated with ART early in the course of infection did not prolong the time to viral rebound following analytical treatment interruption of ART (ATI).
- Therapeutic vaccination had no impact on the size of the HIV reservoir as measured in peripheral blood CD4+ T cells.
- The size of the HIV reservoir was not correlated with the time to viral rebound following ATI.
- The study emphasized the importance of placebo controlled trials in assessing time to rebound following ATI.

Broadly Neutralizing Antibodies (bNAbs)



bNAbs facilitate antibody-dependent cell cytotoxicity (ADCC)

VRC01 study in early treated Thais

Crowell, Colby, Anonworanich, 2017 IAS

Future strategies:

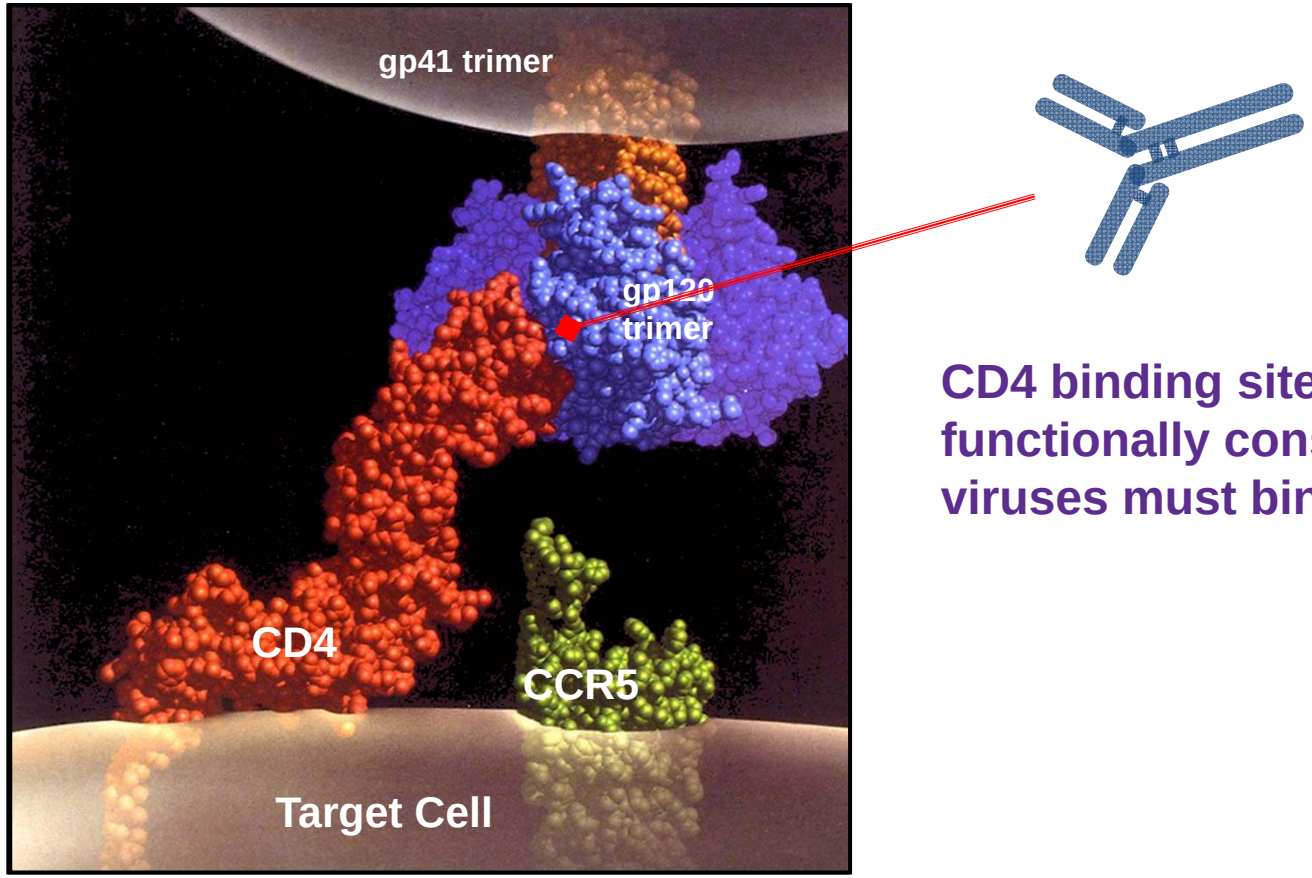
- Combination bNAbs
- Long-acting bNAbs
- Novel delivery platforms

Klein, J Virol 2015; Barr, NEJM 2016; Scheid, Nature 2016 Caskey, Nature Med 2017; Hessell, Nature Med 201
Liu, Science 2016; Nishimura, Nature 2017; Pardi, Nature Communications 2017; Gardner, Nature 201

Broadly Neutralizing Antibodies (bNAbs) Against HIV

- HIV-infected individuals have considerable difficulty making bNAbs *in vivo*.
- However, we have little difficulty in producing these monoclonal antibodies *ex vivo* from cloned B cells of HIV-infected individuals.
- Thus, there is considerable interest in the employment of passive transfer of monoclonal bNAbs for the prevention and treatment of HIV infection.

VRC01 Binds gp120 CD4bs and Blocks Viral Attachment to CD4

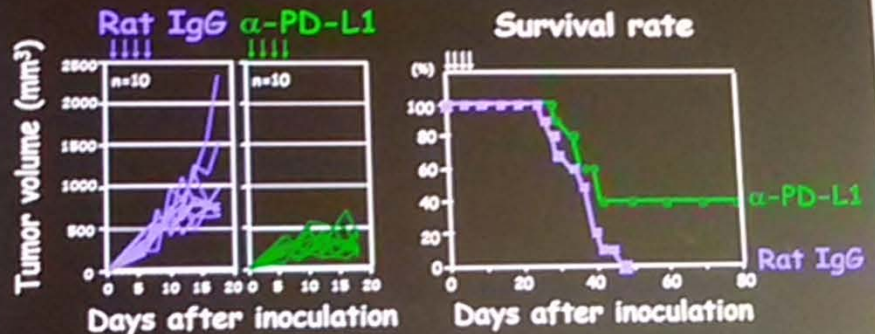


CD4 binding site on gp120 is functionally conserved: All viruses must bind CD4

Inhibition of tumorigenesis of P815/PD-L1 by anti-PD-L1

Iwai et al. PNAS 2002

P815/PD-L1→DBA/2



Human anti-PD-1 antibody

Established by Human immunoglobulin Tg mice
(Xenogenic mice: Medarex: May 9, 2005)

Subclass: IgG4S228P
mutant IgG4 (S228P) stabilizes the protein and reduces ADCC.

KD = 2.6 nmol/L

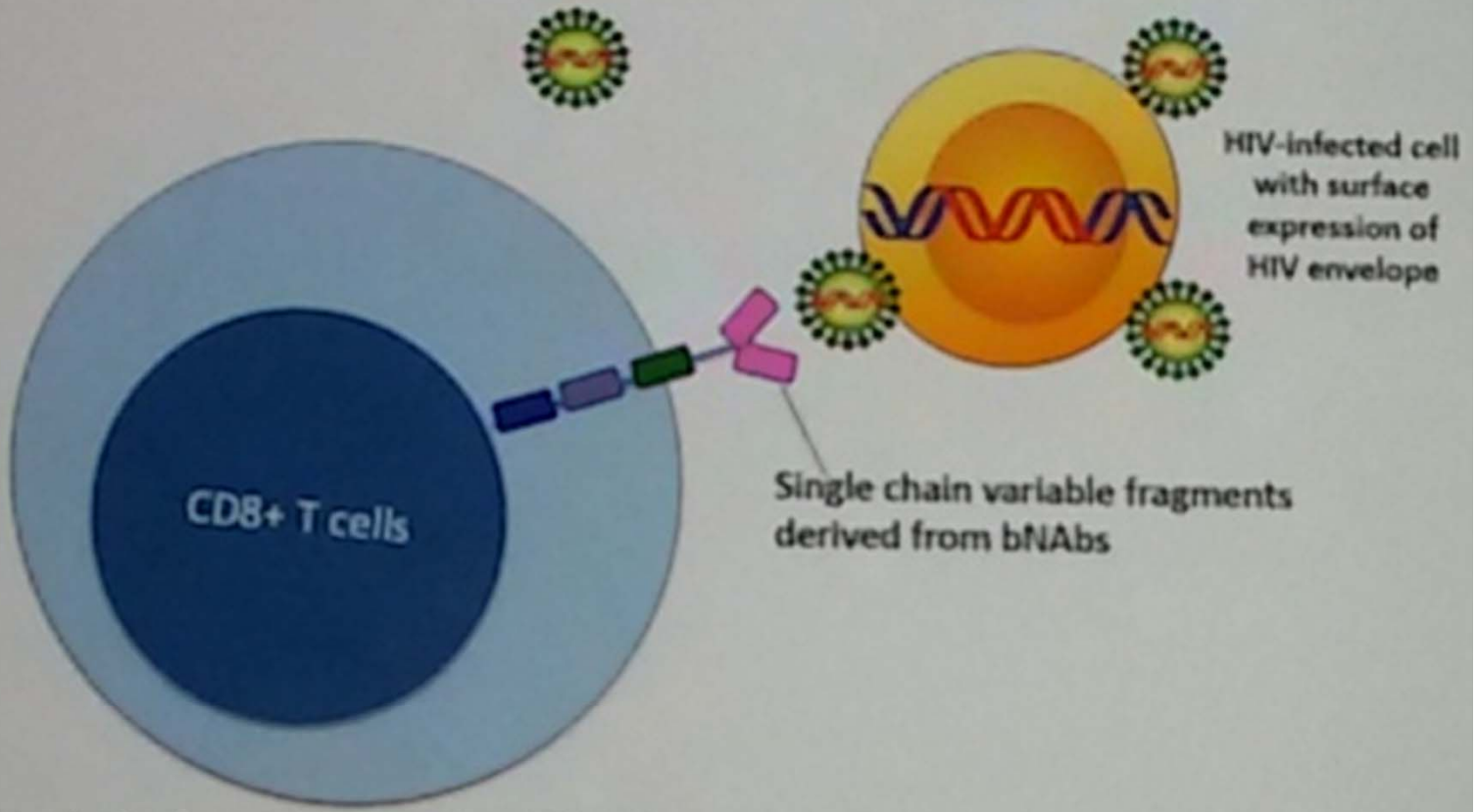
Paradigm shift of cancer therapy by anti-PD-1 treatment

1. Less adverse effects because of no direct damage on normal cells
2. Effective for a wide range of tumors (more than 200 clinical trials)
3. Long-term effects to responders after 6-month treatment

Cancers approved for PD-1 Ab therapy

- 2014 melanoma
- 2015 lung cancer
- 2016 renal cancer
- 2016 Hodgkin's lymphoma
- 2016 head and neck cancers
- 2017 urothelial cancer

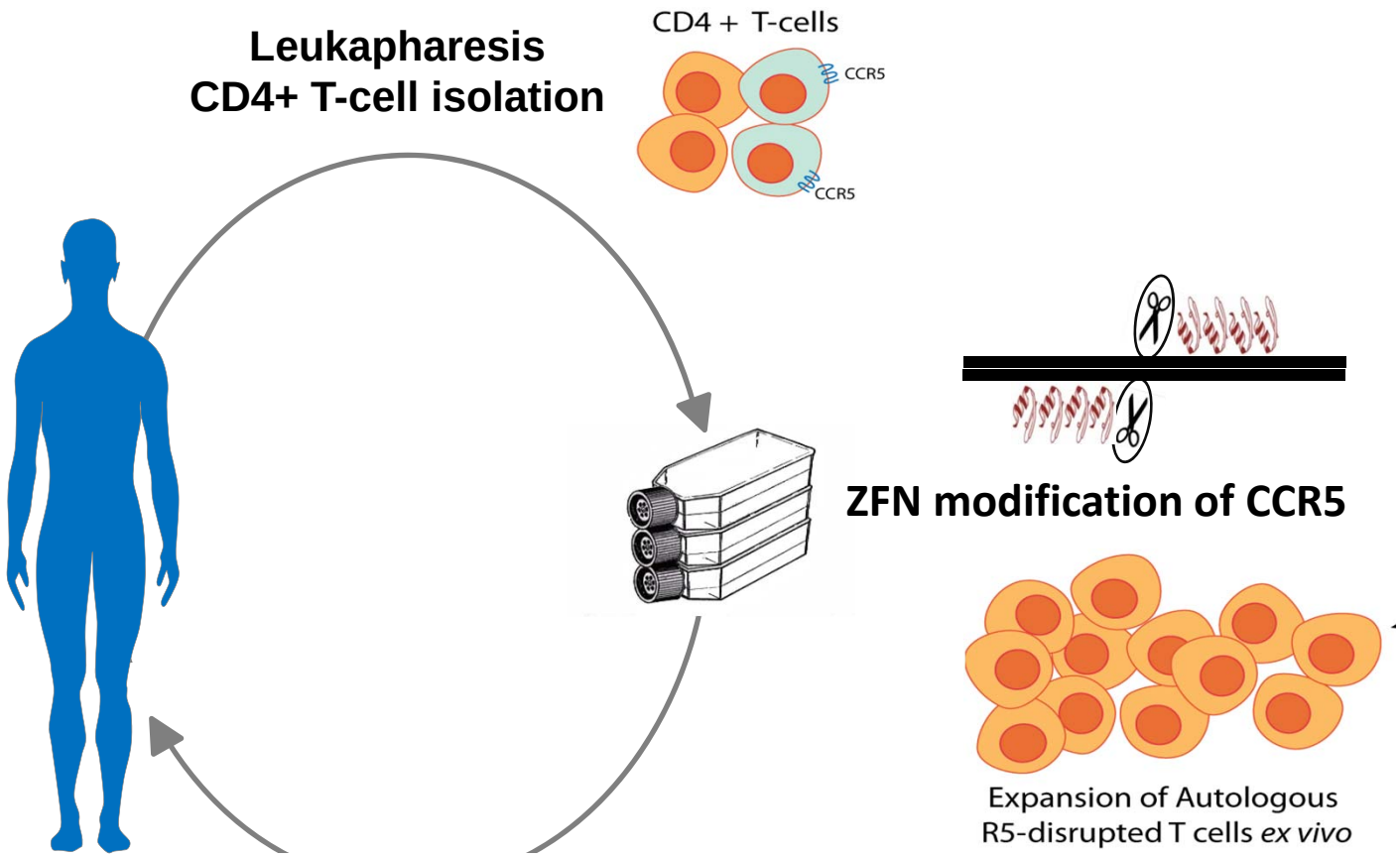
Genetic Engineered T cells: Creating Killer T Cells



Modified from a slide by Dr. Thor Wagner (U Washing

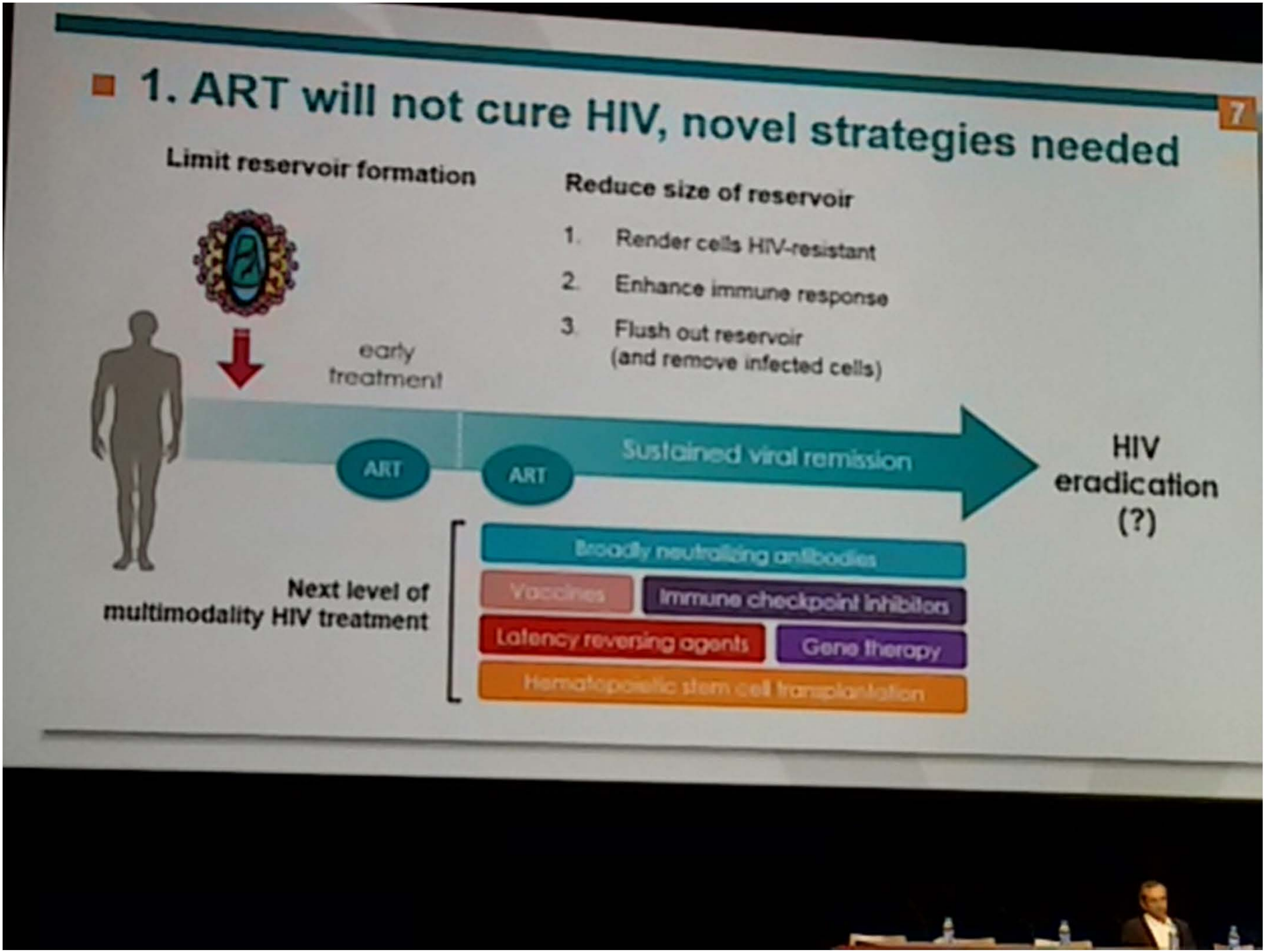
Hale and Wagner, Mol Ther 2017; Ali, J Virol 2016; Liu, J Virol 2016; Hale, Mol Ther

Gene therapy to eliminate CCR5



+ cyclophosphamide

The Berlin patient: CCR5 negative stem cell transplantation


1. ART will not cure HIV, novel strategies needed
7
Limit reservoir formation
Reduce size of reservoir
1. Render cells HIV-resistant
2. Enhance immune response
3. Flush out reservoir (and remove infected cells)
early treatment
ART
ART
Sustained viral remission
HIV eradication (?)
Next level of multimodality HIV treatment
Broadly neutralizing antibodies
Vaccines
Immune checkpoint inhibitors
Latency reversing agents
Gene therapy
Hematopoietic stem cell transplantation

Take Home Message

- HIV infection is associated with a myriad of hematological abnormalities.
- HIV infection should be considered in the assessment of patient presenting with any type of cytopenia.
- Successful ART may reverse or lessen the severity of cytopenia (represents the degree of immunosuppression).
- Due to better outcome after HAART era, NHL should be taken into consideration in diagnostic workup of HIV infected patient with lymphadenopathy.
- Future direction in prevention and treatment of HIV infection will be supported by rapidly emerging field in immunotherapy.

Thank You