For the International Course on Translational Hepatology:

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"Viruses and Cancer"

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All Viruses Known to be Involved in the Origin of Cancers Form Persistent Infections.-----

but what if some viruses cause cancer but are not persisting?? We wouldn’t know!

Human Viruses Involved in Malignancies

- Retroviruses (HTLV-1,HIV*)
- Herpesviruses (EBV,HHV-8)
- Papillomaviruses (HPV-16, -18, etc.)
- Hepatitis B and C viruses*
- Merkel Cell Polyomavirus?

* HIV-1 and HCV involvement in cancer are indirect, i.e., produce affects on UNINFECTED cells to become neoplastic.

The Mechanisms of viral carcinogenesis are by far best understood with retroviruses-both animal and human (HTLV-1).

All are DIRECT i.e.,act on the infected cell and provide clear cut evidence of causation but with variable mechanisms.

Naturally Occurring Retroviruses

- **Leukemia viruses (oncoretroviruses) cause leukemia/lymphoma in chickens (ALV), mice (MuLV), cats (FeLV), cattle (BLV), monkeys (STLV-I), apes (GaLV, STLV-I), koalas (KoRV) and humans (HTLV-I).**

- **Lentiviruses cause immunodeficiencies in cats (FIV), cattle (BIV), monkeys (SIV), humans (HIV).**

- **Retroviruses also cause anemia (FeLV in cats, EIAV in horses), neurologic disease (visna in sheep, HTLV-I and HIV-1 in humans), autoimmune diseases (HTLV-I, FeLV), breast cancer (MMTV in mice), and osteopetrosis (ALV in chickens)**

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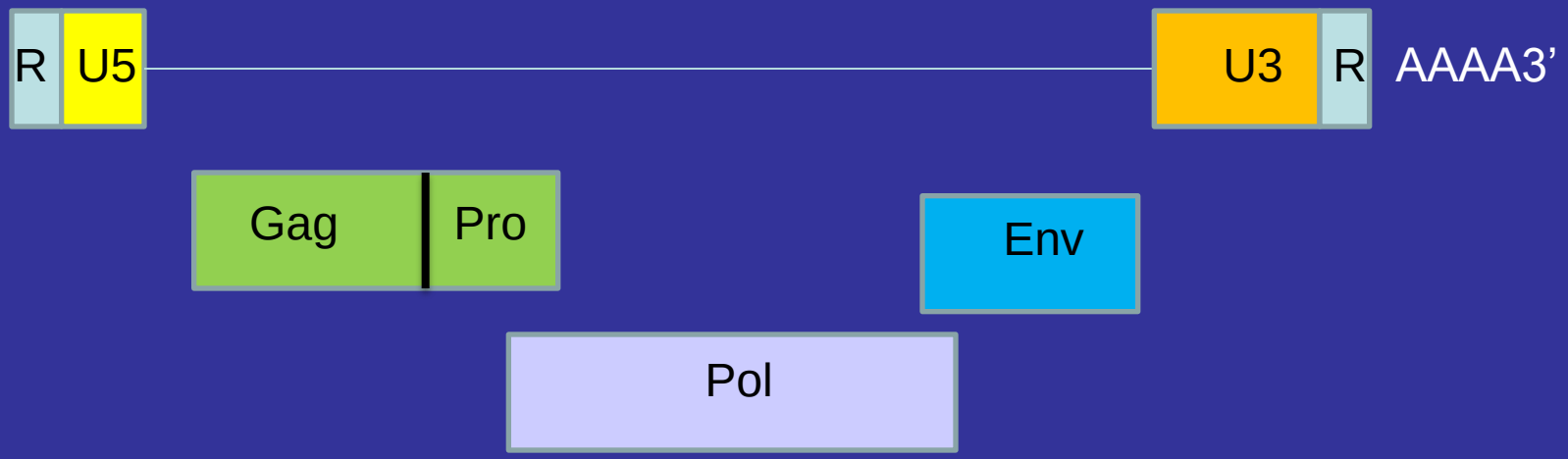
Genomic structure of simple retroviruses



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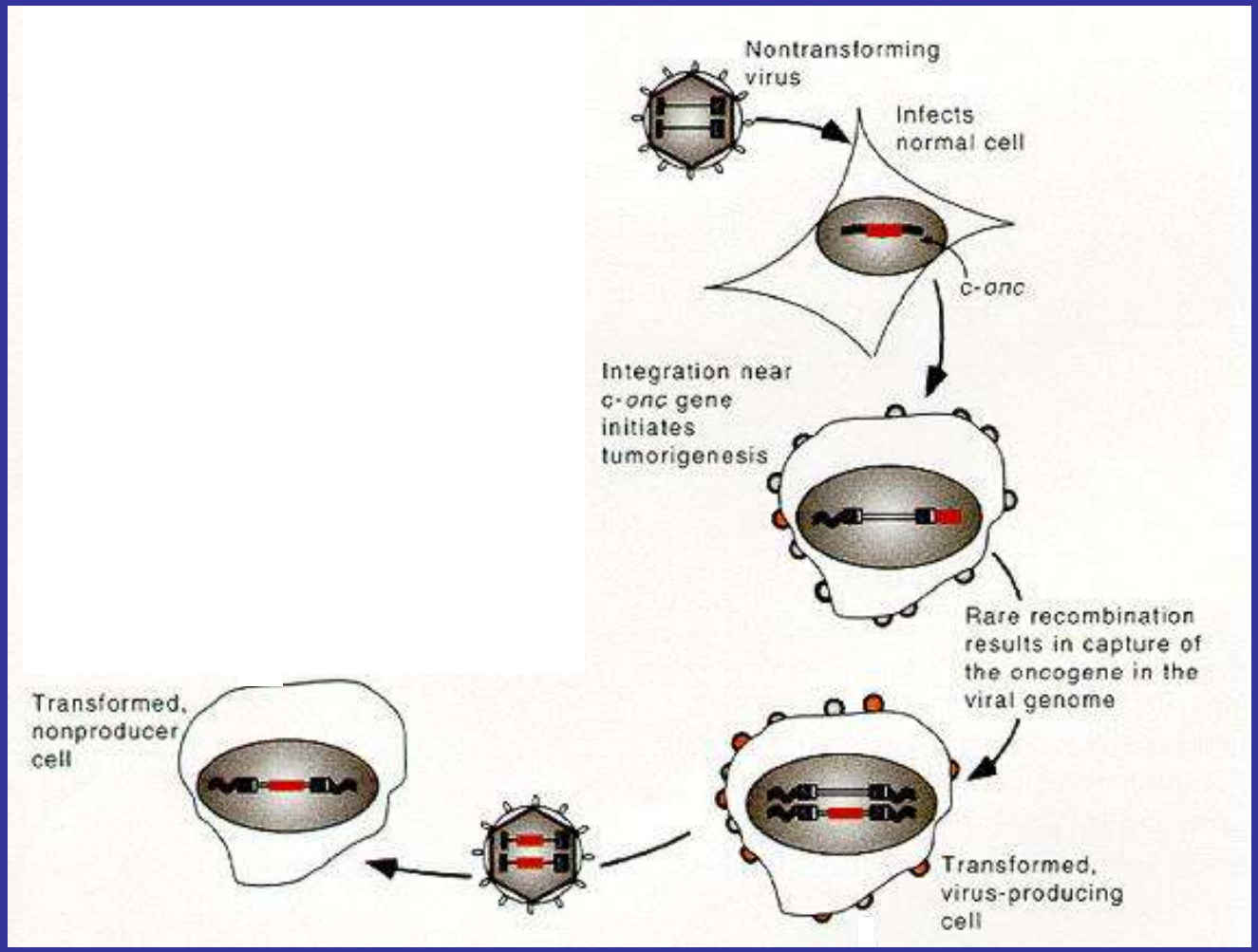
Some mechanisms of retroviral tumorigenesis: 1) Transduction of transforming genes

- Virus captures a cellular gene (proto-oncogene)
- Rare and usually requires high viral replication
- Occurred in transmission of GaLV from gibbon ape to woolly monkey, captured *sis* resulted in fibrosarcoma
- Occurs in FeLV, *fes* captured, results in multifocal fibrosarcomas
- Occurs in MuLV, captures *ras*, sarcomas and lymphomas result
- Occurs in ALV, captures *src,* sarcomas result
- Not documented yet in humans
- Viruses are generally defective—therefore a one time event

Generation of acute transforming virus by oncogene capture



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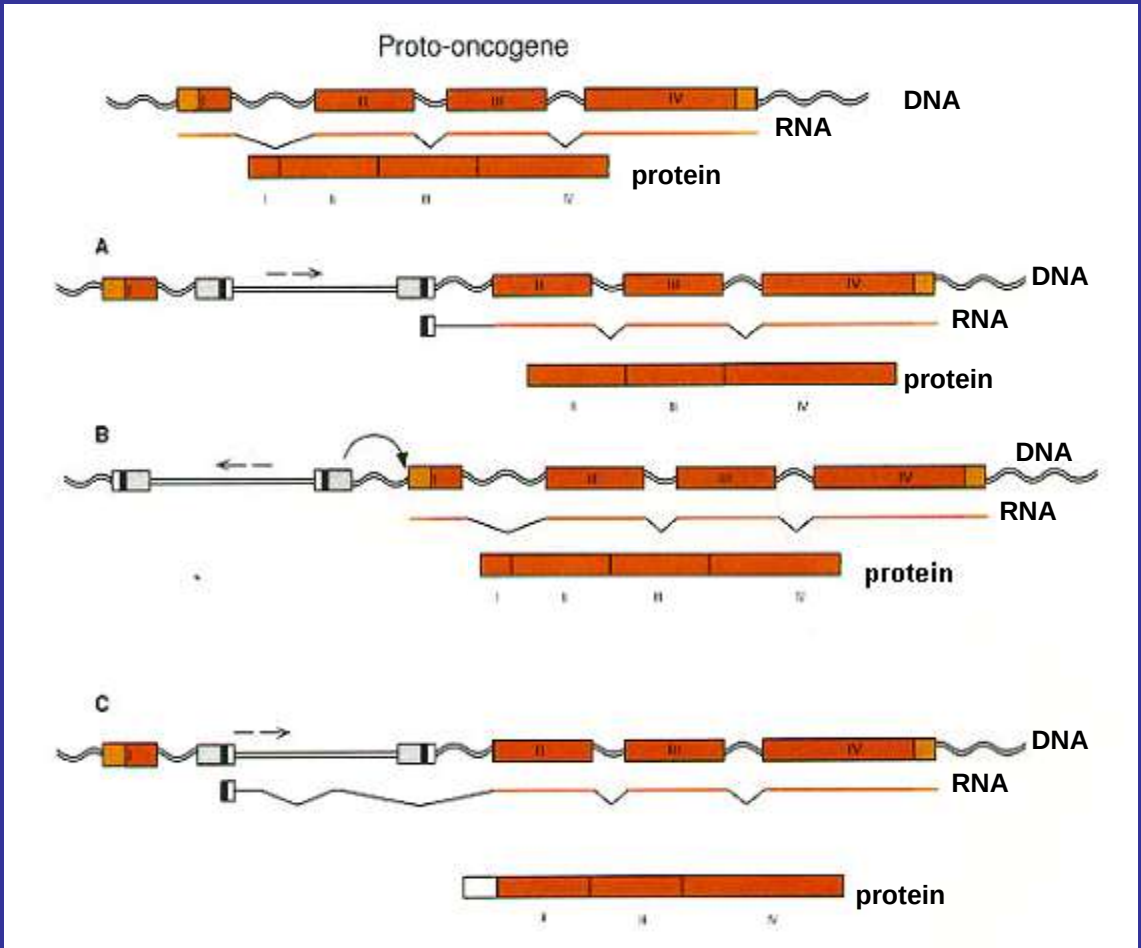
Some mechanisms of retroviral tumorigenesis: 2) Insertional mutagenesis

- Provirus inserts near proto-oncogenes (c-myc is typical, but a great variety of gene targets have been reported and likely happened in a human)
- Dysregulates expression or causes gain or loss of function mutation
- Most common mechanism of retroviral leukemogenesis (e.g., FeLV, MuLV, GaLV, ALV)
- Integration is semi-random, so high viral replication is needed

Types of insertional mutagenesis



Normal transcription, wild type cellular protein

Viral promoter insertion - protein truncated and transcription dysregulated

Viral enhancer insertion – transcription dysregulated

Read-through transcripts - virus-cell fusion protein, transcription dysregulated

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SIMPLE RETROVIRUSES exceptions to the rules:

- Friend mouse erythroleukemia virus-has some virions with a truncated envelope gp55, which acts like erythropoietin. (no insertional mutagenesis).

- Human-- given a MuLV vector for gene therapy (LTR-----inserted gene-----LTR) got acute T cell leukemia. Insert was near transcriptional regulator, LMO2. No viral replication was needed! This was a surprise.

- Another surprise: why no leukemia with HIV? Though not a simple retrovirus it replicates extensively and almost randomly integrates.

Some mechanisms of retroviral tumorigenesis: 3) virus encodes a growth factor mimetic

- Very rare. The example is Friend MuLV envelope protein in which an internal deletion within *env* gene resulted in gp55 erythropoietin mimetic.

- After sufficient time and erythroid replication, due to genetic or epigenetic changes, leukemia can become independent of gp55.

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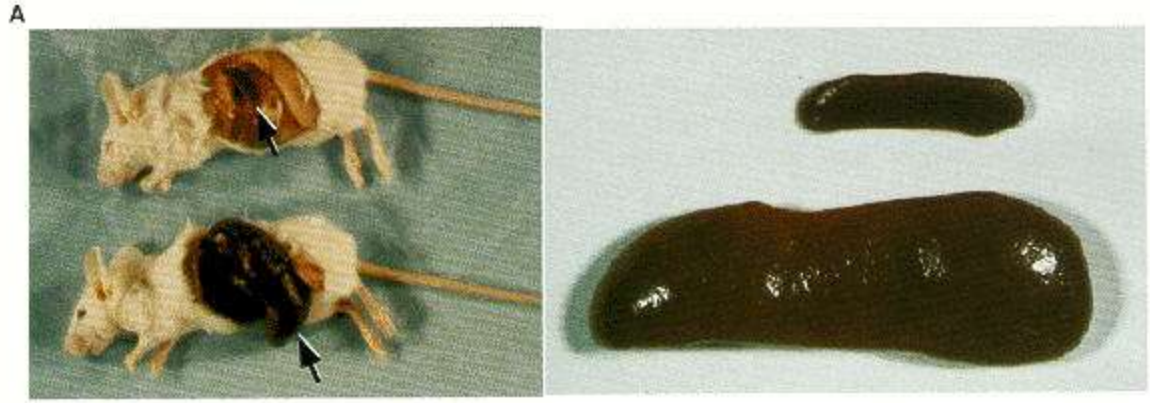

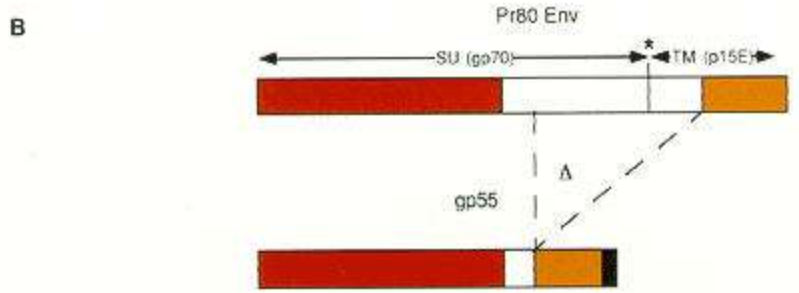

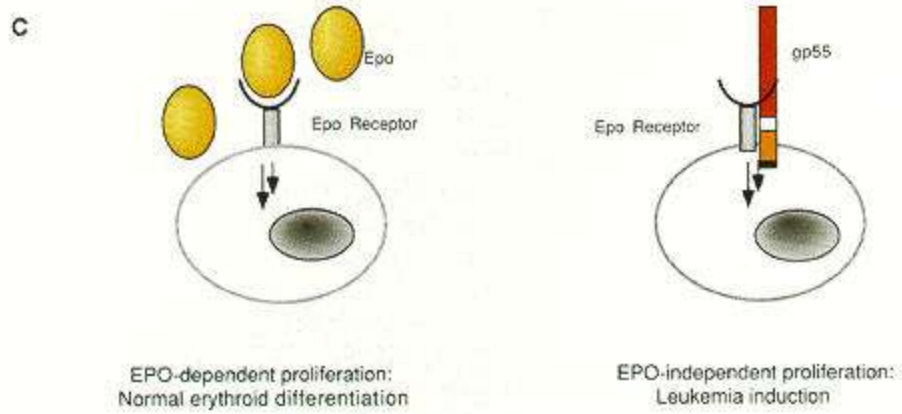

Friend leukemia virus envelope protein has erythropoietin activity

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Some mechanisms of retroviral tumorigenesis:
4) virus encodes an extra gene—not a cellular derived oncogene—this now shifts the discussion to human viruses

- The extra gene encodes a non-stuctural protein that can directly stimulate proliferation and promote genetic instability (HTLV, BLV)
- The extra gene is not cell derived (not a proto oncogene) or its origin is unknown

- BLV and HTLV do not appear to require cofactor

COMPLEX RETROVIRUSES: contain extra gene(s)

- Extra gene(s) may cause growth promotion and/ or increase of genetic instability.

  LTR---gag-pol-env-extra gene(s)---LTR

- Examples:

  HTLV-1 (the prototype),

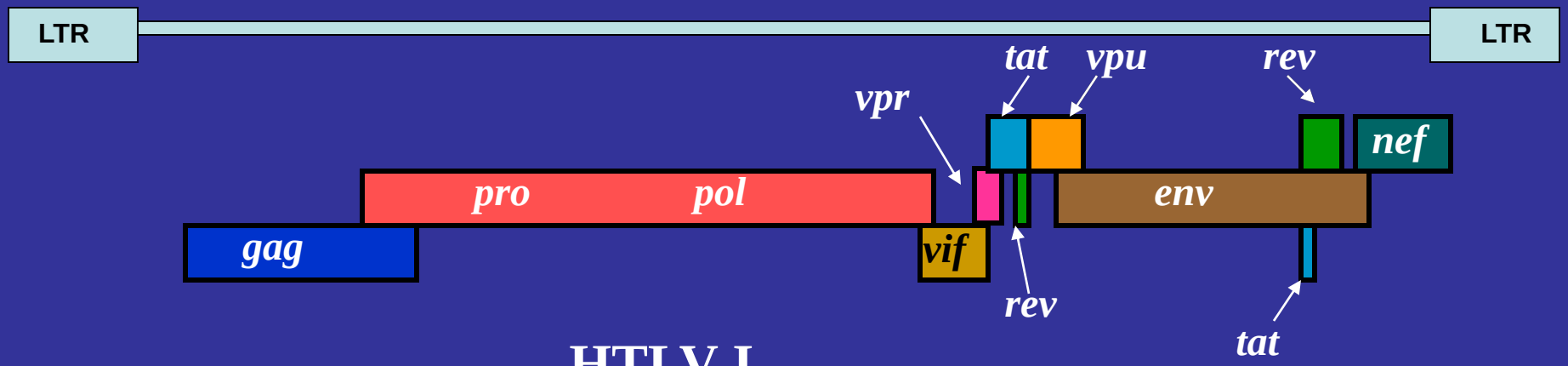
  Bovine Leukemia Virus (BLV),

  Simian T-Cell Leukemia Virus (STLV-1)

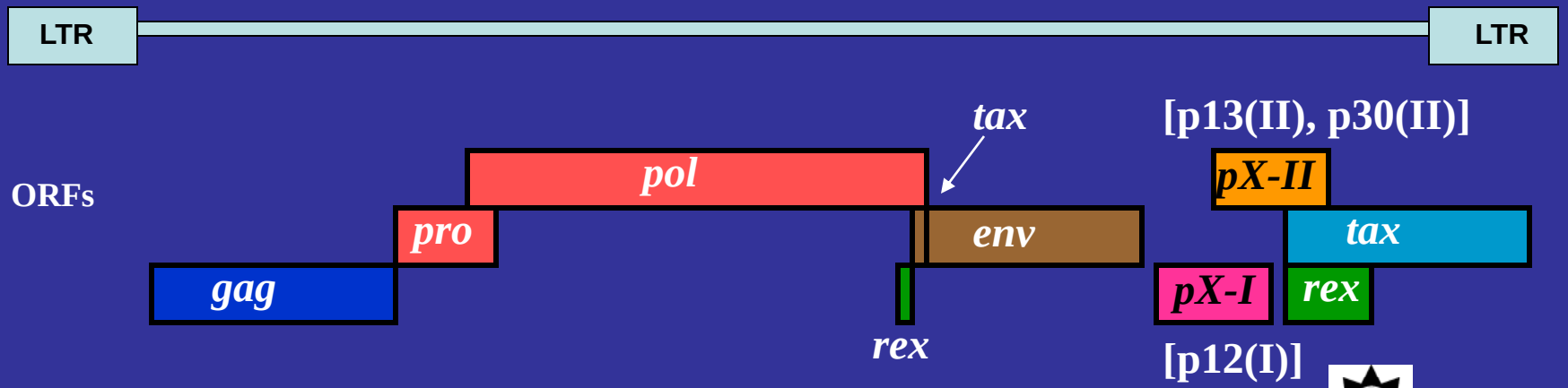
*Lentiretroviruses such as HIV also have complex genomes but no known growth promoting genes.

Genomes of Human Retroviruses

HIV-1



HTLV-I



The Beginning (the 1970ies): Remarkable Biases

- **“Infectious diseases are over in the industrialized world, therefore . . .”**
- **“Retroviruses do not infect humans and there are many reasons for this . . .”**
- **“No viruses cause cancer in man.”**

Circa 1970-80

Why Prejudices Were So Strong Against Even the Possible Existence of Human Retroviruses

Decades of repeated failed attempts

High replication in animals (i.e., easy to find if they exist)

Little evidence in primates (until late 1970s)

Human sera is lytic for many animal retroviruses

IN THE EARLY 1980S THE BIASES WERE SHATTERED

- Viruses shown to be the cause of 15 to 20% of human cancers.
- Retroviruses discovered in humans, shown to cause some leukemia's and neurologic diseases HTLV-1 1980 and HTLV-2 1982.
- One of the great pandemics of history (AIDS) appears and is caused by another retrovirus.

Introduction of HTLV-1 AND HTLV-2

- **First human retroviruses** to be discovered (1980 and **1982)**
- **Only human leukemia causing virus** (HTLV-1)
- **Best evidence for a viral cause of any human cancer** (clear-cut epidemiological linkage, numerous animals get leukemia by similar retroviruses, immortalizes and causes chromosomal instability in its target CD4 T cell- the same cell type of the leukemia it causes, and virtual proof of its causative role in that the provirus is found CLONALLY integrated in the leukemic cells, & no cofactor involved.

Introduction of HTLV-I (cont.)

- Causes adult T cell leukemia (ATL) but latent phase is many decades, once the leukemia appears rapid progression to death within 3-6 months. 1-10% lifetime risk of leukemia in infected people
- Causes tropical spastic paraparesis (TSP), a multiple sclerosis-like disease, autoimmune disorders (uveitis, etc.)
- ATL leukemic cells have clonally integrated viral DNA, indicating original malignant cell was infected
- Transmitted by blood, sex, and mother to infant
- About 30 million people infected

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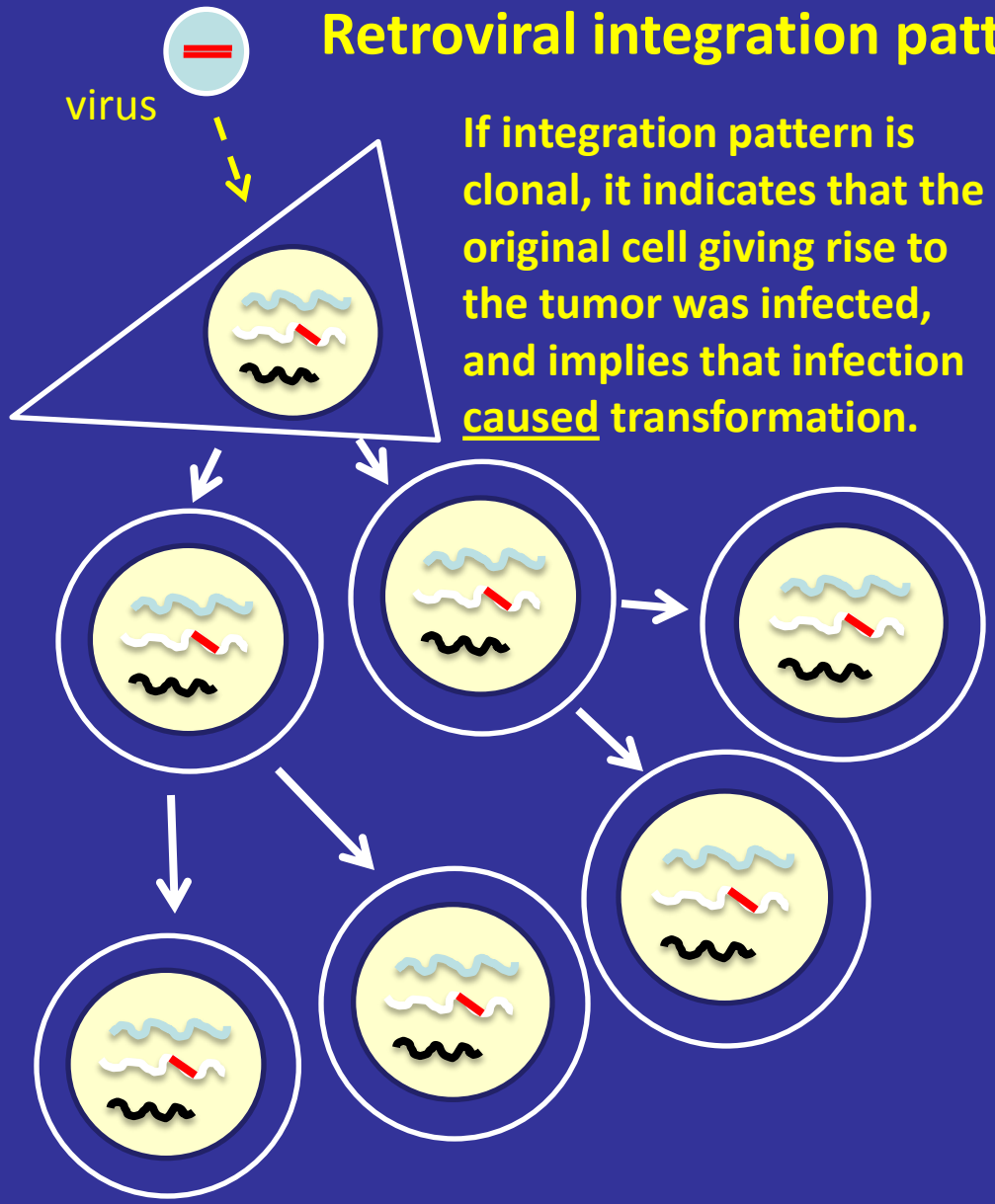
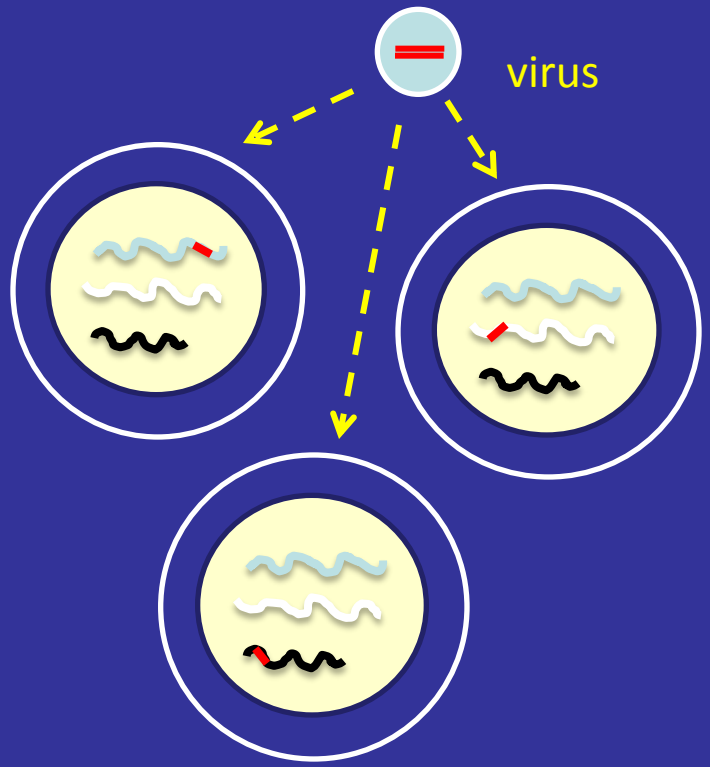
Retroviral integration patterns indicate causality



If integration pattern is clonal, it indicates that the original cell giving rise to the tumor was infected, and implies that infection caused transformation.

If integration pattern is random, it indicates that infection occurred only after transformation.



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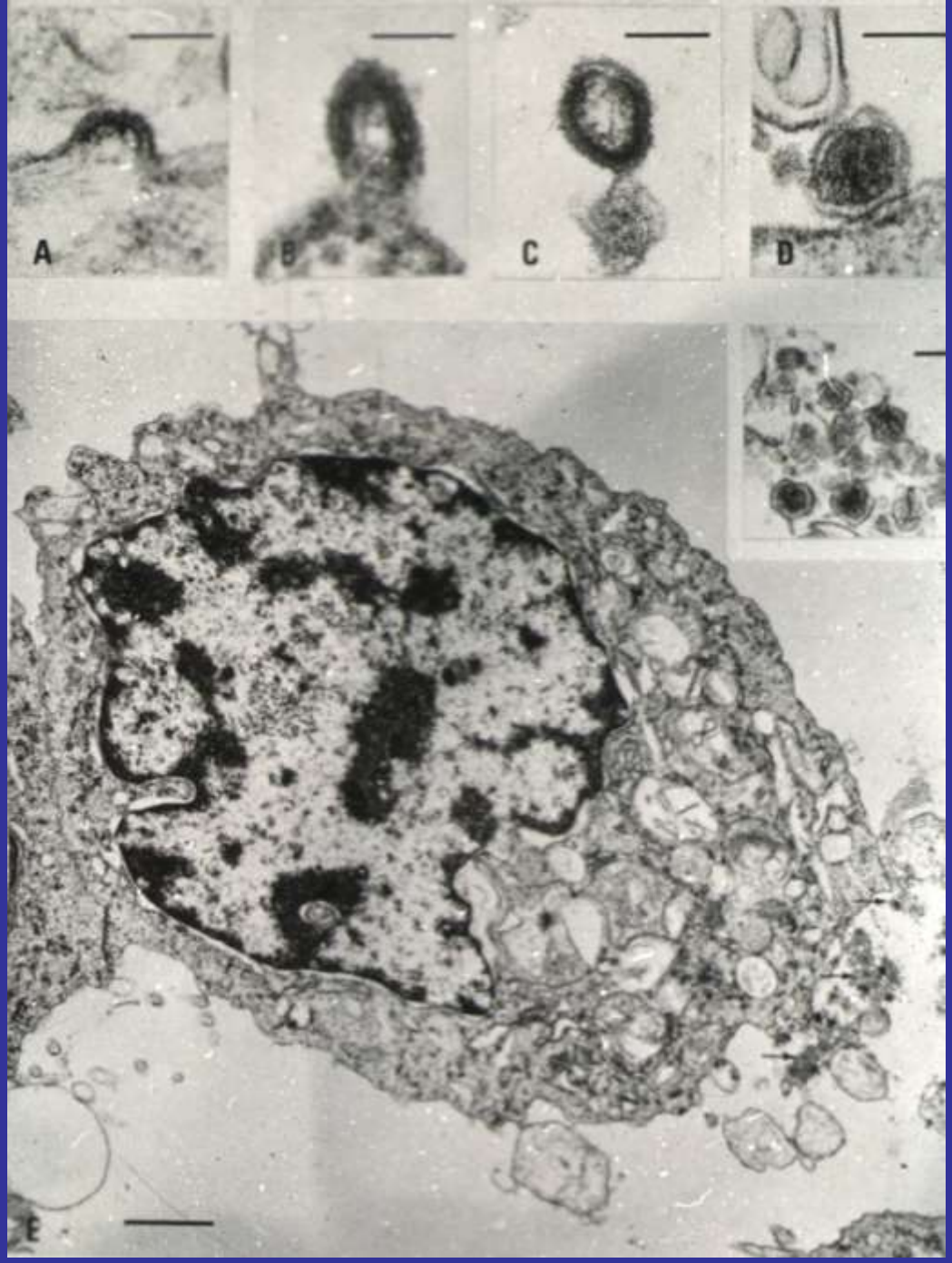
A
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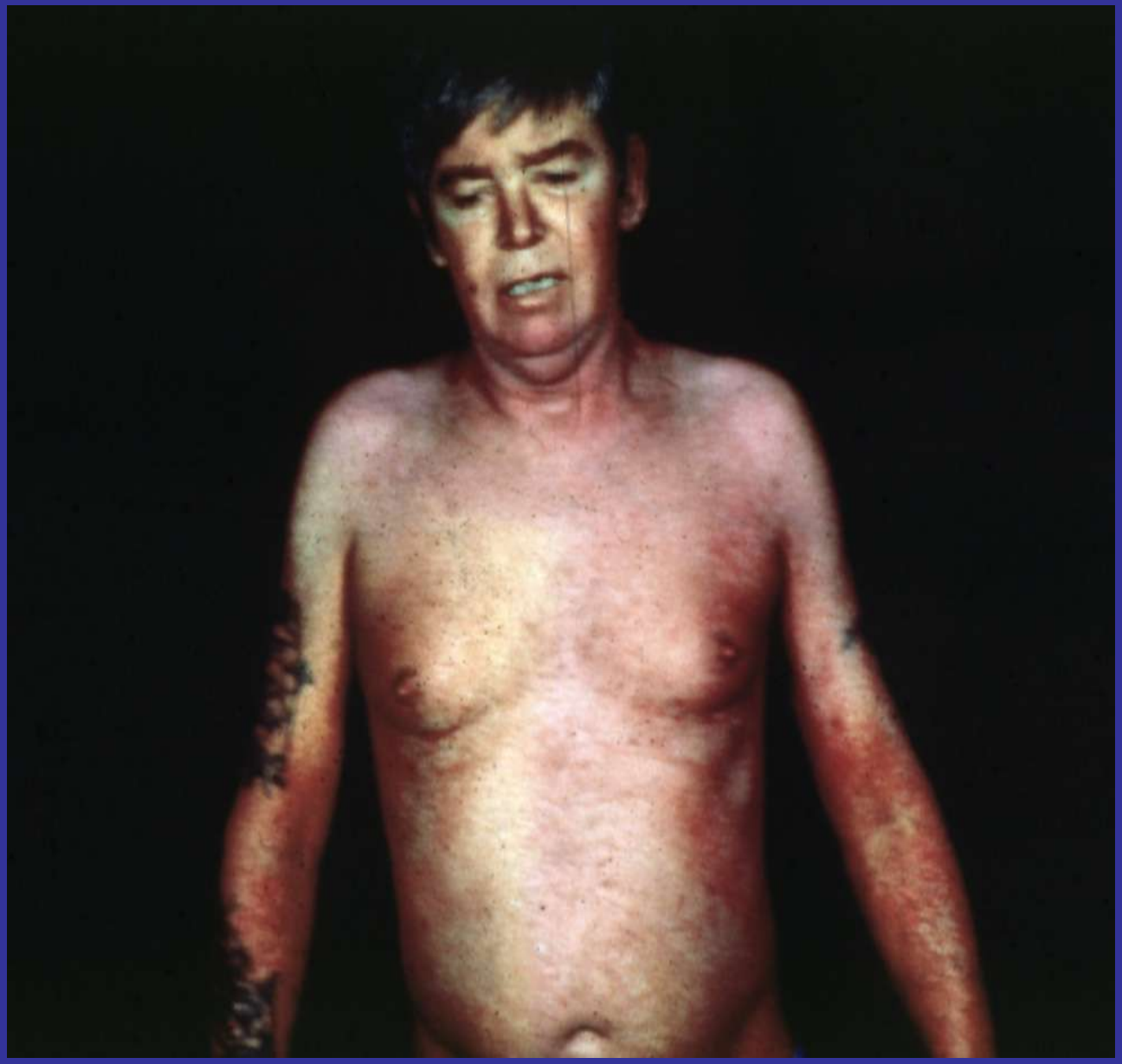
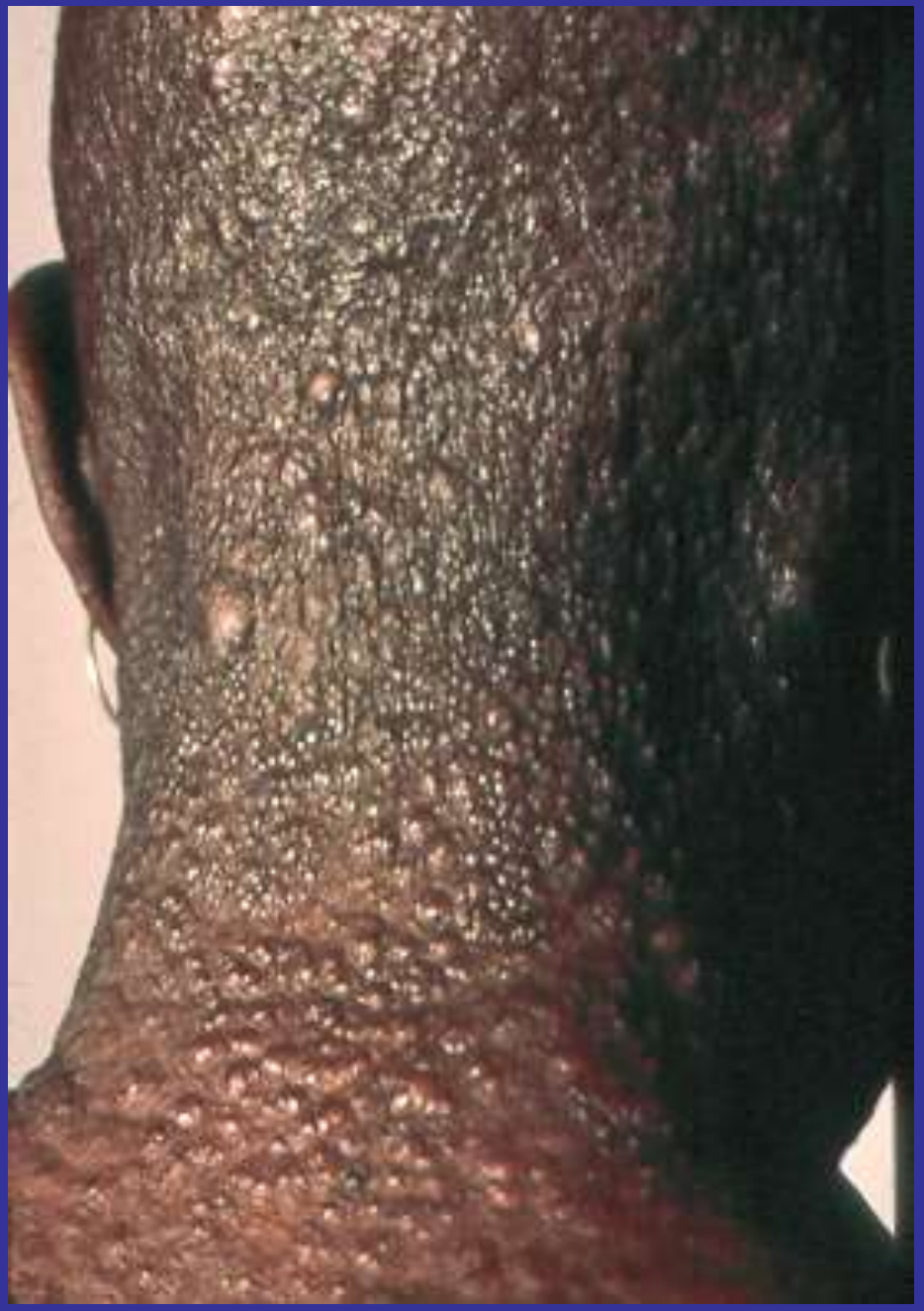


ATL (Adult T Cell Leukemia)

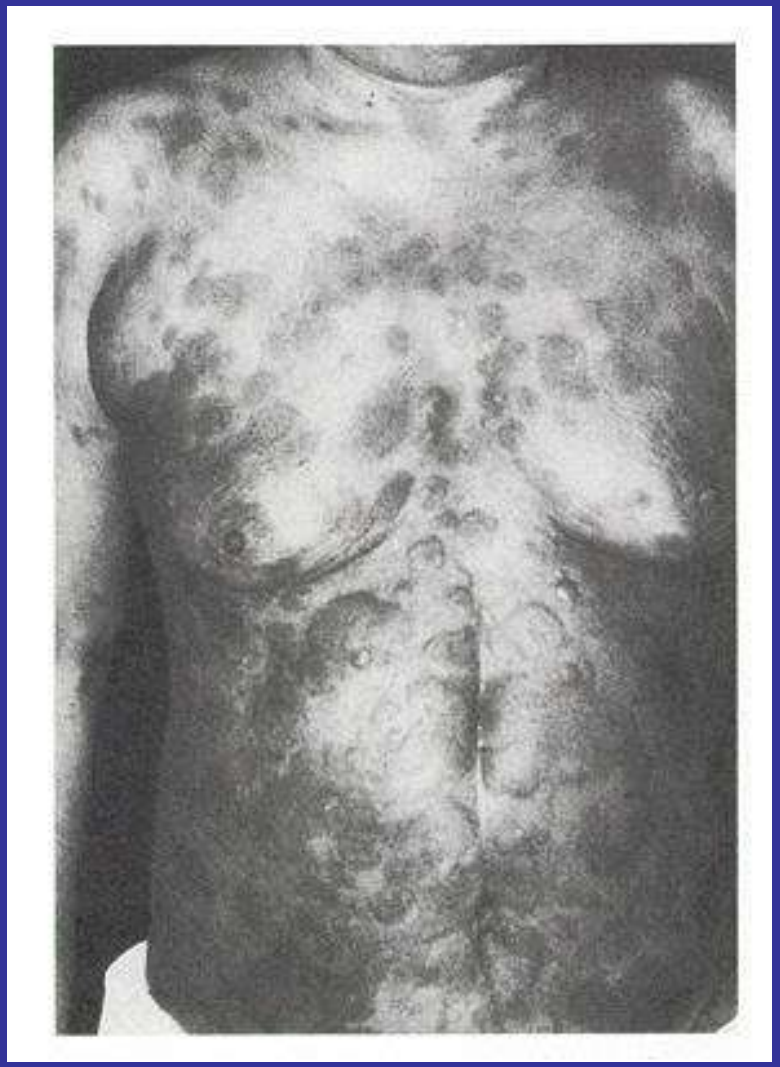
- Described in 1977 by Takatsuki and coworkers before virus was known.

- Typically skin lesions of variable kinds, hypercalcemia (frequent) with lytic lesions of bones, lymphadenopathy, diagnostic morphology of leukemic cells, and an extremely aggressive clinical course.

- Almost always involves the mature CD4+ T Cell.



Adult T cell leukemia caused by HTLV-I



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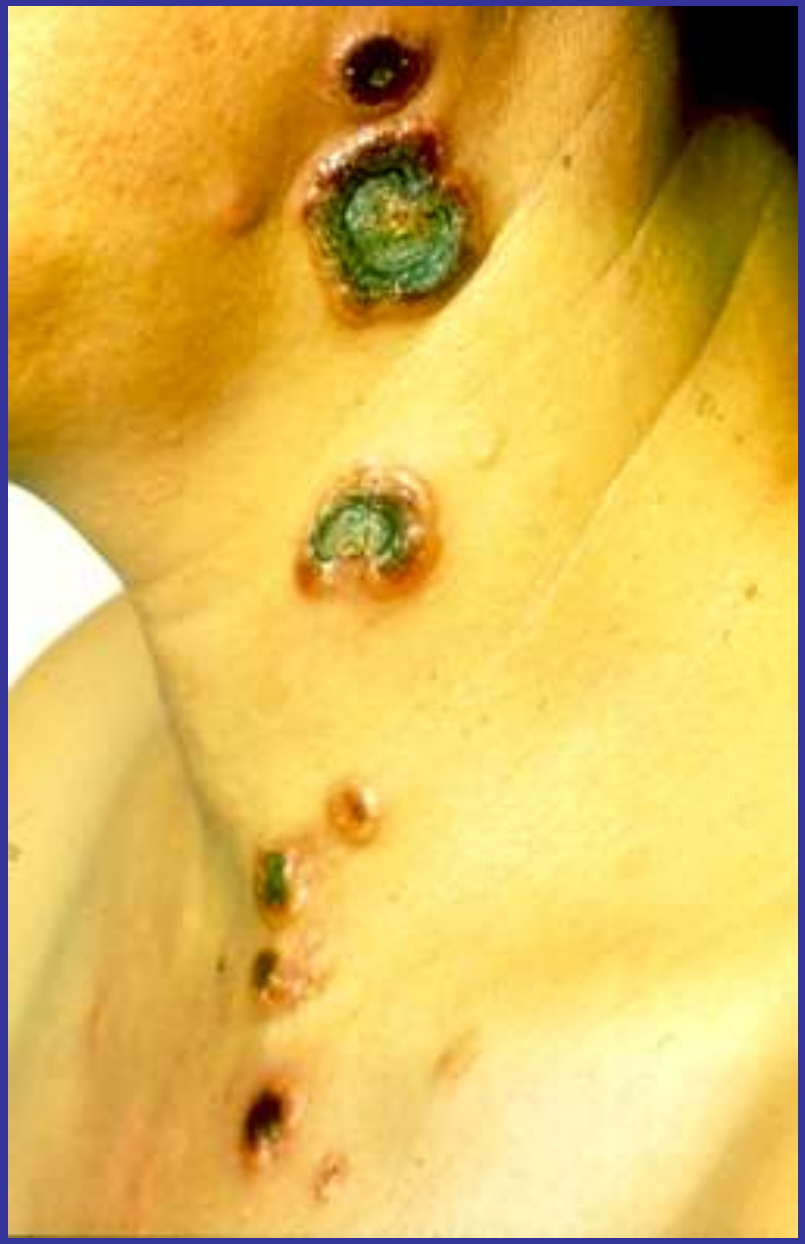
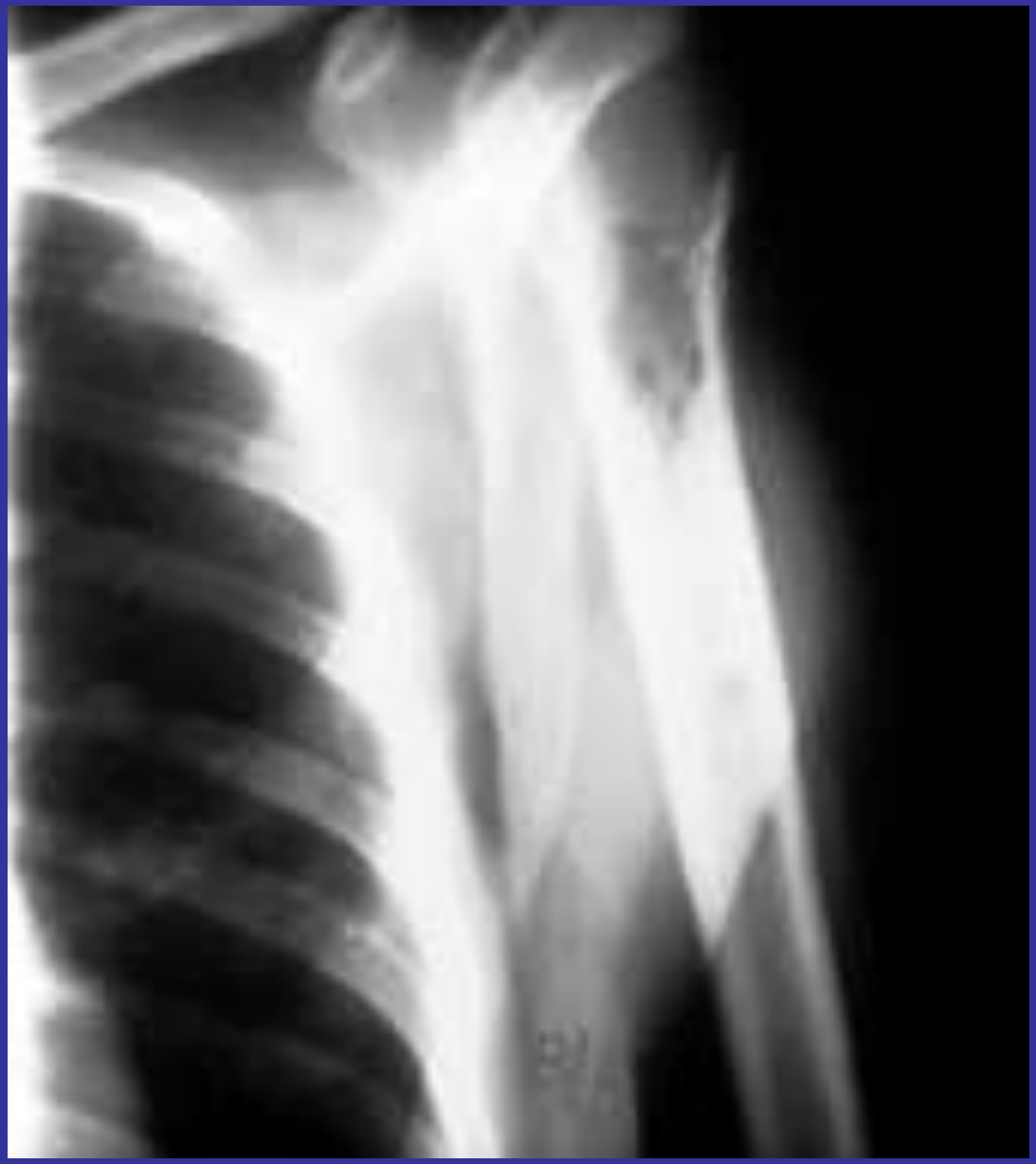
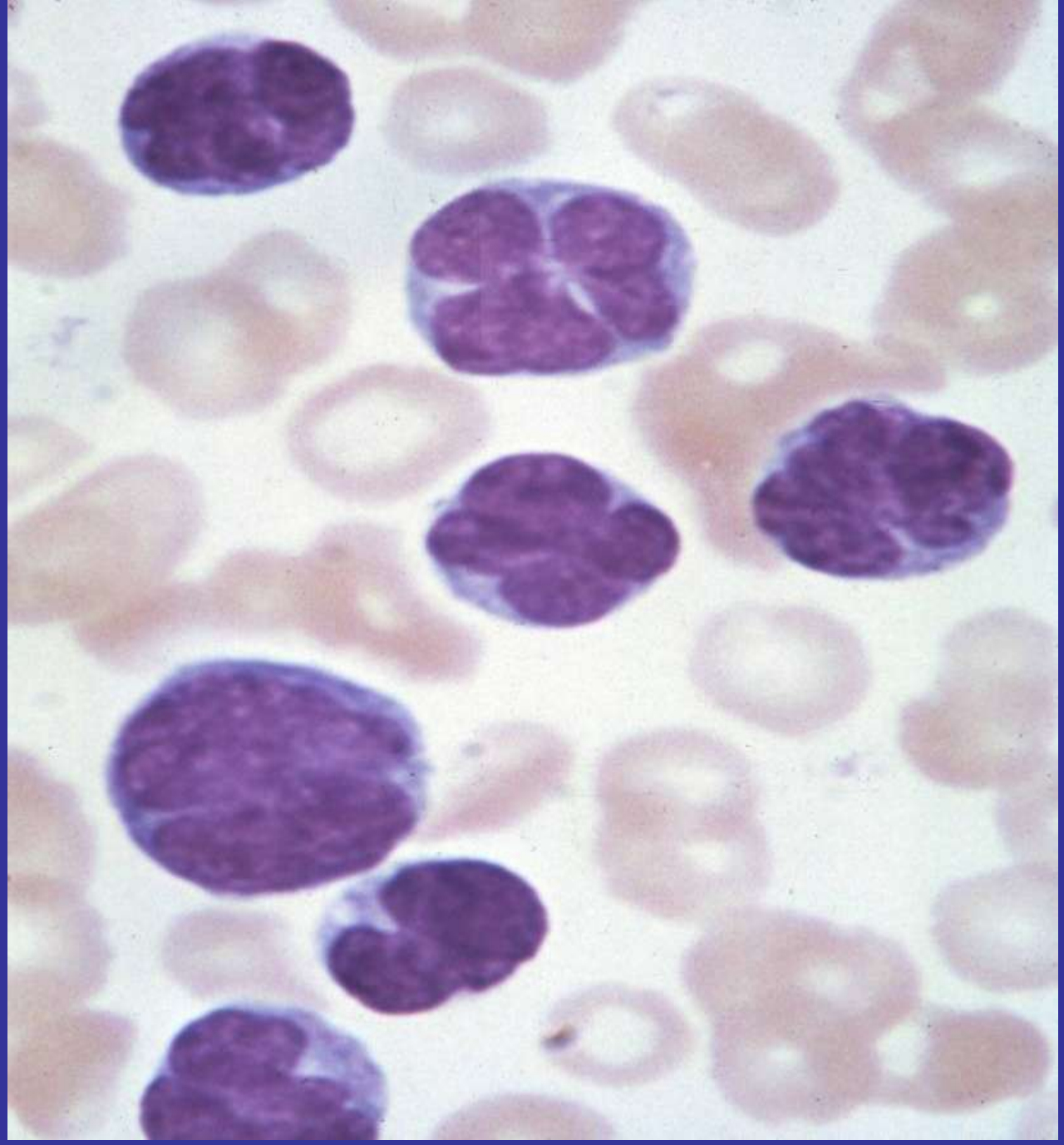
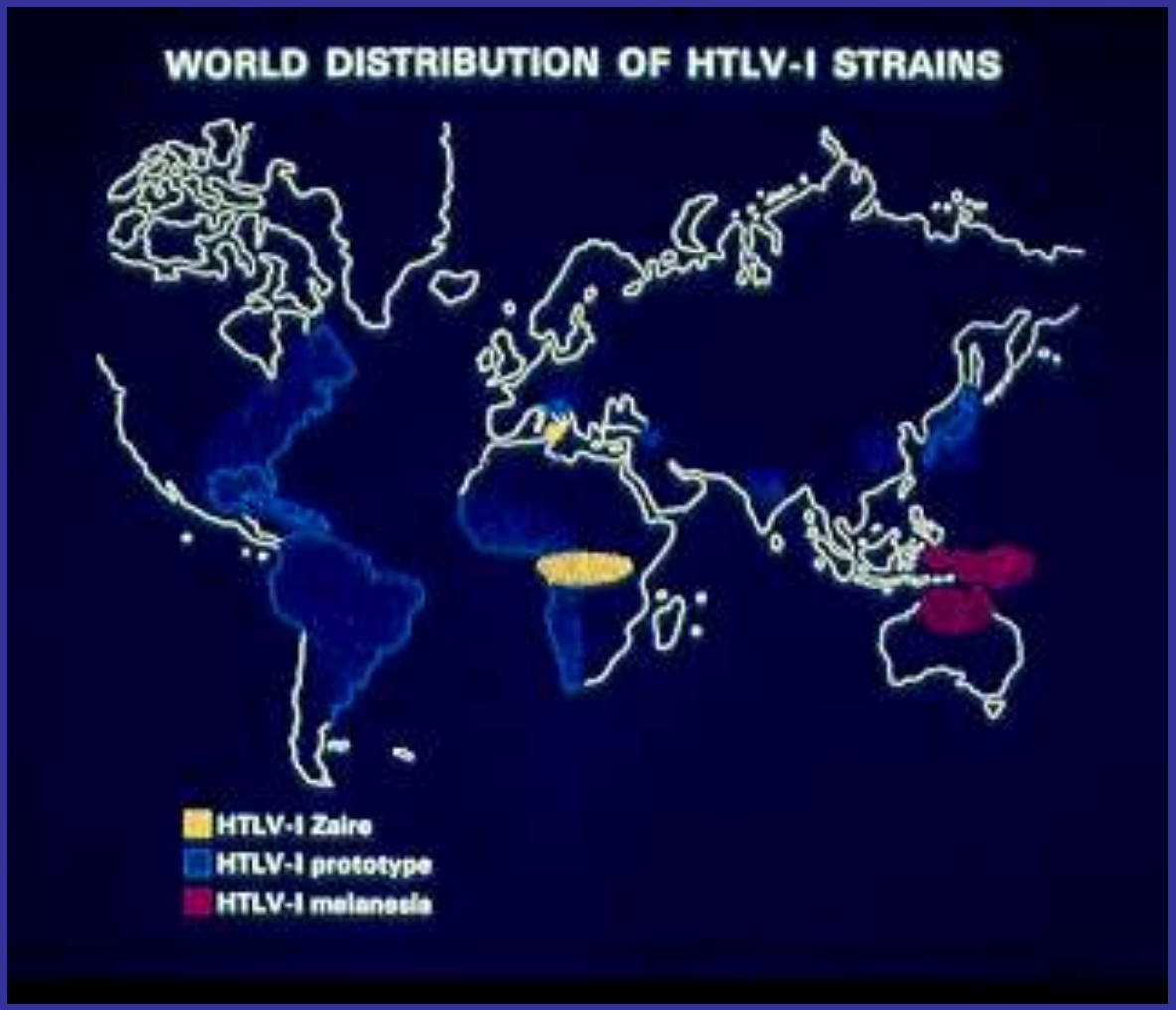






PREVALENCE OF HTLVs IN DIFFERENT REGIONS


WORLD DISTRIBUTION OF HTLV-I STRAINS
HTLV-I Zaire
HTLV-I prototype
HTLV-I melanesia

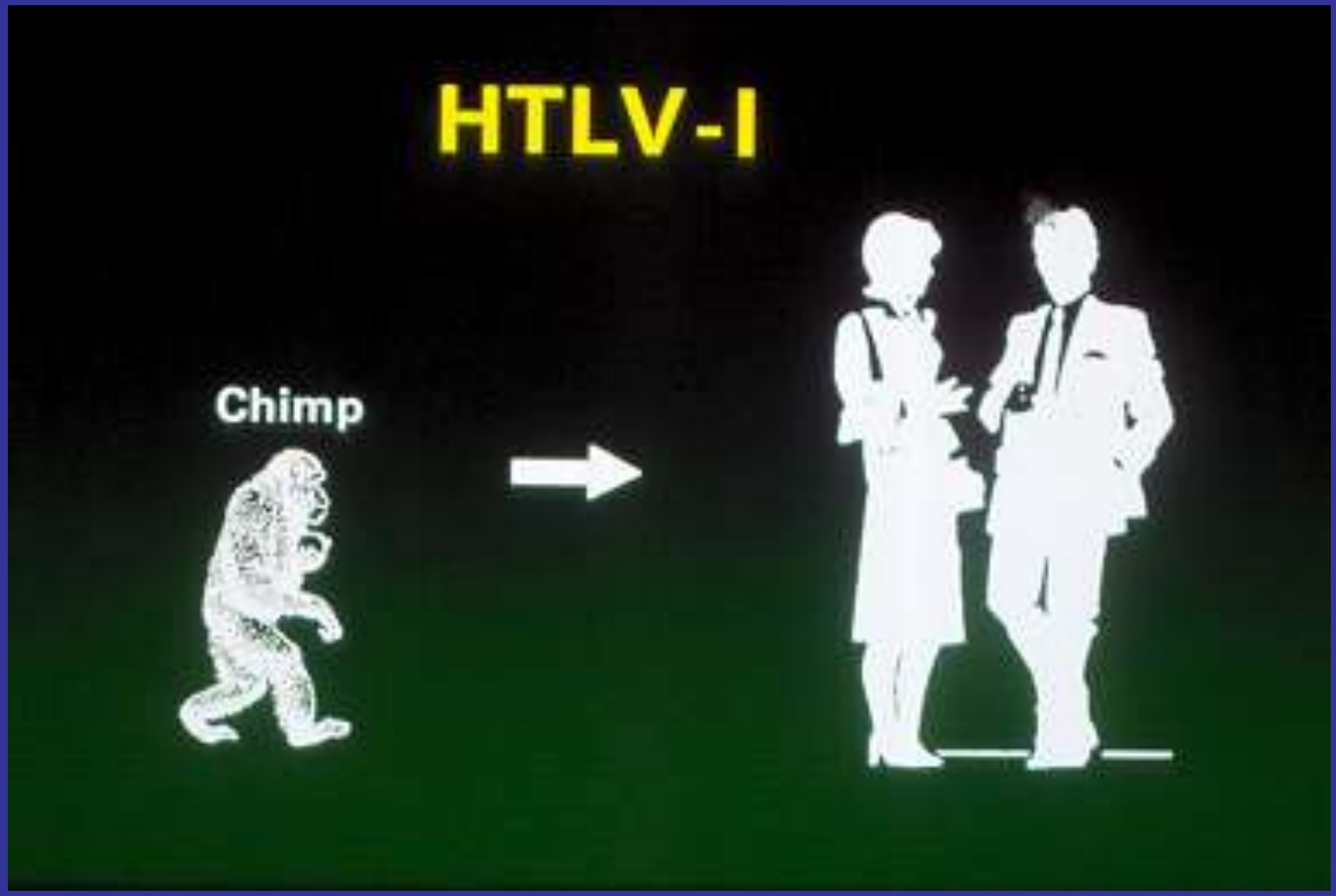

HOKKAIDO
0%+
HONSHU
Shimane Prefecture 0%+
Kanagawa Prefecture < 1%+
Nagoya Area 2%+
Kobe-Osaka Area 4%+
SHIKOKU
Nagasaki Area 16%+
Uwajima Area 9%+
ATL ENDEMIC AREAS
Kagoshima Area 15%+
KYUSHU


HTLV IN THE CARIBBEAN
AND SOUTH AMERICA
FLORIDA
BAHAMAS
POSITIVE CASES
POSITIVE NORMALS
CUBA
DOMINICAN
REPUBLIC
VIRGIN ISLANDS
JAMAICA
HAITI
PUERTO RICO
DOMINICA
GUADELOUPE
MARTINIQUE
ST. LUCIA
ST. VINCENT
BARBADOS
GRENADA
NICARAGUA
COSTA RICA
TRINIDAD
VENEZUELA
PANAMA
GUYANA
SURINAM
COLUMBIA
CHILE
BRAZIL
ECUADOR


NEW GUINEA
PAPUA
NEW GUINEA
SOLOMON
ISLANDS
MAROVO
RENDOVA
GUADALCANAL
RENNEL
BELLONA
VANUATU
AUSTRALIA

Why is HTLV-1 Relatively Important in Some Unexpected Places, for examples, London? Miami??

ANCIENT ORIGIN OF HTLV-1


HTLV-I
Chimp

Survival of HTLV-1 depends upon propagation of the integrated DNA provirus BECAUSE THERE IS VERY LITTLE VIRAL REPLICATION

Therefore, survival depends on T cell proliferation.

Adult T cell Leukemia is the occasional accidental consequence.

What are the molecular pathogenic mechanisms of HTLV-I?

Tax

1. **Transforms T cells: inhibits apoptosis, promotes cell proliferation**
2. **Transcriptional activator and repressor- activates NFκB, CREB/CRE (dysregulates cellular gene expression)**
3. **Binds to p16INK4A, an inhibitor of CDK4/6 (dysregulates cell cycle)**
4. **Inhibits topoisomerase I (promotes genome instability)**
5. **Causes mislocation of spindle assembly checkpoint factors hsMAD1 and hsMAD2 (loss of mitotic checkpoint)**
6. Inhibits expression of DNA Polymerase Beta

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THUS—HTLV-1 PATHOGENESIS

- Is critically dependent on the Tax protein in initiation and early stages of pathogenesis

- But, unlike HPV-16,-18 NOT in the maintenance of the transformed cell which can have many distinct but not necessarily common molecular lesions.

- So it is different from CML, Burkitt Lymphoma, and some other cancers with one specific molecular “lesion”,but more like what may be for most cancers that may have more than one path to neoplasia.

Breakthrough in Therapy (2006 to 2010)

- P. Gill, USC and A. Bazarbachi, U. Lebanon
- Interferon-alpha, AZT, and Aso4 compounds
- From a disease with a 3 to 6 month survival from the time of diagnosis to apparent complete remissions.
- Singular most important work since the discovery of HTLV-1.

‘LEGACY” OF HTLV-1

- FIRST HUMAN RETROVIRUS AND ONLY KNOWN LEUKEMIA VIRUS.
- ONLY TUMOR VIRUS NOT KNOWN TO **NEED** ONE OR MORE CO-FACTORS.
- CAN PROVIDE CONTINUOS GROWTH IN THE LAB. OF ANY MATURE CD4+ OR CD8+ T CELLS.
- PROVIDED THE IDEA AND TECHNOLOGY FOR THE DISCOVERY OF HIV.
- MAY PROVIDE MOLECULAR INSIGHTS INTO THE MECHANISMS OF LEUKEMOGENESIS FOR SOME OTHER TYPES OF LEUKEMIAS as well as SOME NEUROLOGICAL and AUTOIMMUNE DISEASES.

HIV as a Non-Tumor Virus That is Tumorigenic, i.e., HIV can Increase the Incidence of Some Cancers and Not Just from Immune Suppression

HIV in Cancer (as a “co-carcinogen

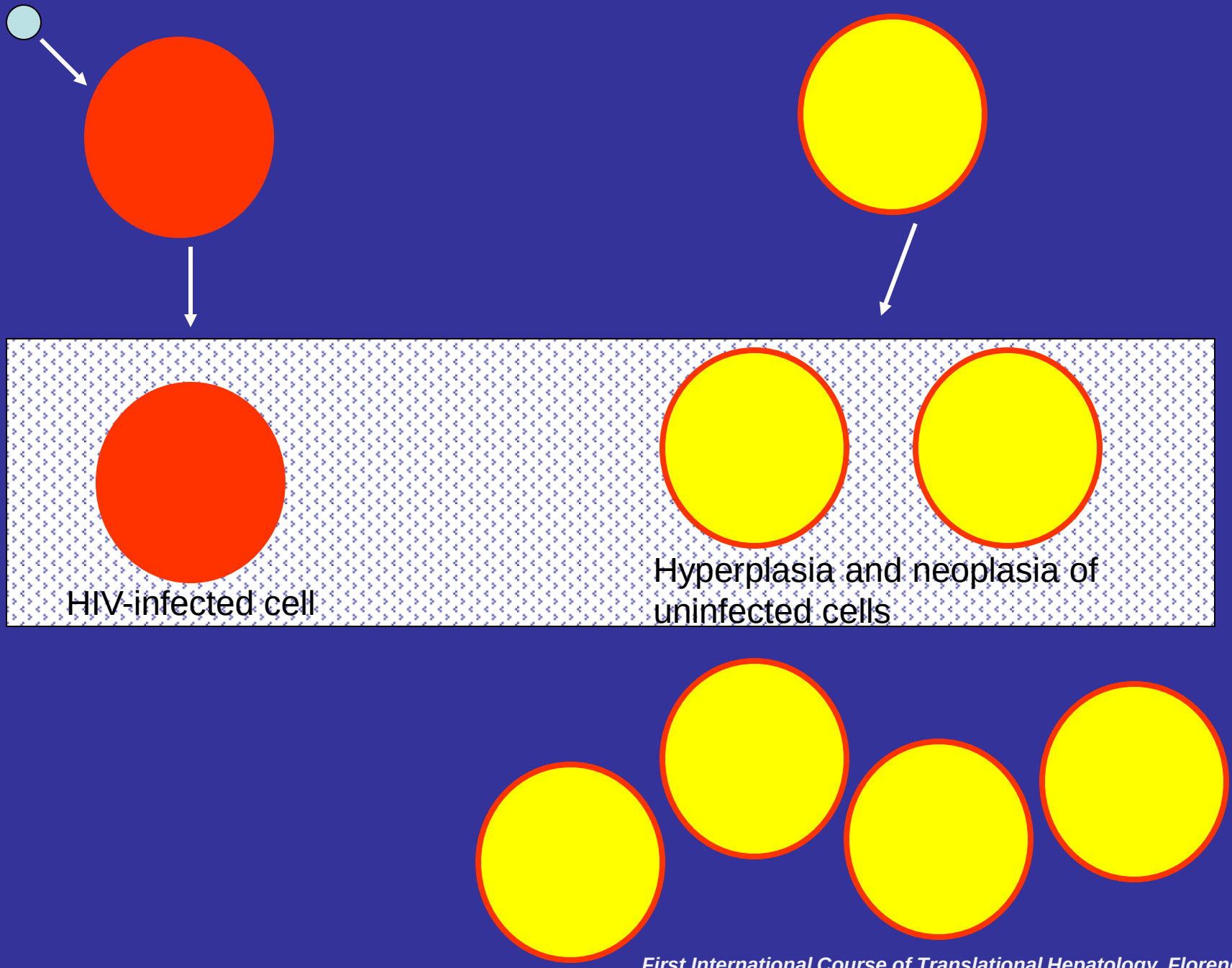
Liver (HBV; HVC)

Lymphoma (EBV; HHV-8)

Kaposi’s sarcoma (HHV-8)

Genital and cervical CA (HPV)

Leiomyosarcoma (EBV in children)


HIV-infected cell
Hyperplasia and neoplasia of uninfected cells

KS and non-Hodgkins B cell lymphoma: The two main (but not sole) cancers markedly increased by HIV.

The co-carcinogenic role of HIV is mediated by the microenvironment

and mainly involves some HIV proteins and cellular cytokines.

4 Models of Viral Tumorigenesis

1. **Virus acts by insertional mutagenesis, does not transform cells directly (MuLV, FeLV, ALV)**
2. **Virus affects cell cycle or lifespan, but does not directly cause tumors (EBV, HHV-8)**
3. **Virus encodes gene(s) that transform infected cells (HTLV, HPV)**
4. **Virus acts very indirectly—affecting the microenvironment and may not necessarily reside in the tumor cells at all . Hard to get exact mechanisms.**

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Human Papilloma Virus

- **HPV-16, 18, and selected other strains are high risk for cervical, anal, and oral cancers.**
- **HIV-1, cigarette smoke components (benzo[α]pyrene) may be co-factors.**
- **Role of integration is somewhat unclear, but may predispose to cancer by stabilizing/enhancing viral transcription.**
- **E6 and E7 proteins contribute directly by dysregulating cellular factors (p53, Rb), are thus direct oncogenes**
- **Thus, like HTLV-1 some HPV strains are clear direct causes of cancers.**

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γ-Herpesviruses

Epstein-Barr virus (Burkitt's lymphoma, B cell lymphoma, some types of Hodgkin's lymphoma, nasopharyngeal carcinoma, infectious mononucleosis)

Human herpesvirus 8 (Kaposi's sarcoma, primary effusion lymphomas, multicentric Castleman's disease)

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EBV

- Etiologic agent in Burkitt's lymphoma (BL), nasopharyngeal carcinoma (NPC), some Hodgkin's disease, yet is present in 95% of normal population. What co-factors lead to tumor formation?
- Association of BL with malaria belt suggests chronic immune stimulation is a co-factor.
- c-myc rearrangement seems to be required for some lymphomas. HIV a cofactor for some EBV lymphomas?
- Tung and croton oils may be cofactors for NPC. They contain TPA, which activates lytic viral replication

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What is evidence that EBV causes tumors?

- **Burkitt's lymphoma onset is predicted by a rise in antibody titers to lytic phase viral proteins**
- **NPC patients have elevated anti-EBV titers**
- **In some forms of Hodgkin's disease, clonally rearranged viral episomal DNA is present**

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HHV-8

- Apparent prevalence worldwide varies from 1-5% in US, UK, Caribbean, India, to 30% in some regions in Italy, to 60-70% in some regions in Africa
- Prevalence 100% in Kaposi's sarcoma (KS), primary effusion lymphoma, Castleman's disease
- Generally does not cause disease- but HIV co-infection is 10,000-100,000 fold risk factor
- Rise in antibody titers, serum viral DNA predicts KS

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HHV-8

- Is present in majority of spindle cells in later KS lesions
- Expression in 90-95% spindle cells in later KS lesions is restricted to a few genes (latency genes); in 2-5% of cells virus is in lytic phase
- Is lost from cultures spindle cells after a few passages

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What is role of HHV-8 in KS?

- **HHV-8 is in all KS patients- suggests strongly that it is necessary.**
- **However, HHV-8 prevalence is much higher than KS incidence- HHV-8 must be very inefficient.**
- **HIV-1 increases risk of KS 10-100,000 fold- HIV is thus a greater risk factor than HHV-8 for KS, but is not absolutely necessary.**
- **HHV-8 infected spindle cells are scarce in early KS lesions, but virtually all spindle cells in late lesions contain HHV-8.**

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Role of HIV in KS: Early Hypothesis (pre-HHV-8 period)

- One major factor in the enormous enhancement of KS development in HIV-1 infected persons is mediated by Tat
- Experimental models (*in vitro* and *in vivo*) showed that Tat increases inflammatory cytokines and angiogenesis

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Tat accelerates KS tumorigenesis in a murine xenotransplant model with cells expressing HHV-8 vGPCR

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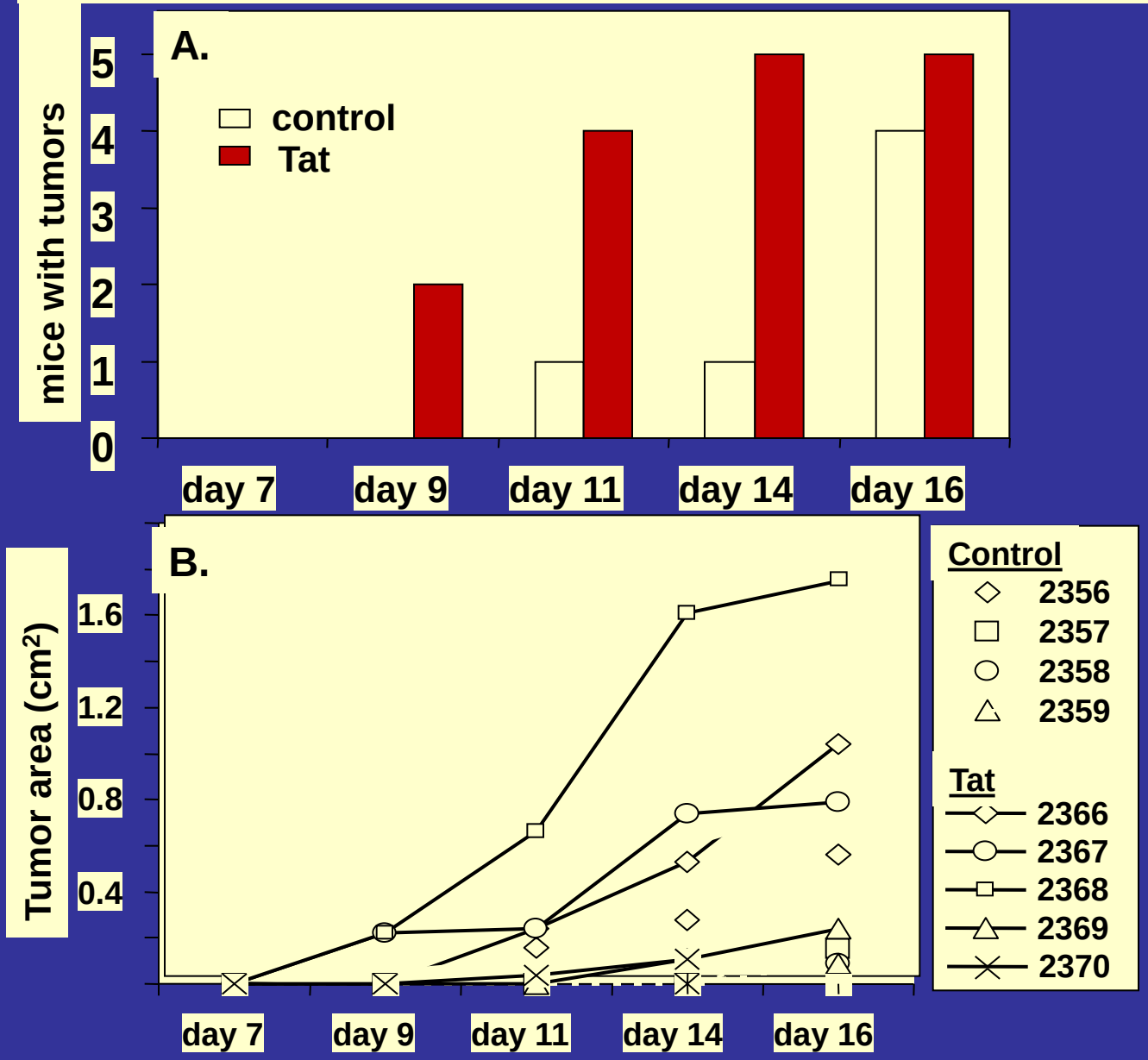
Tat accelerates vGPCR-dependent tumor formation in mouse KS xenotransplant model



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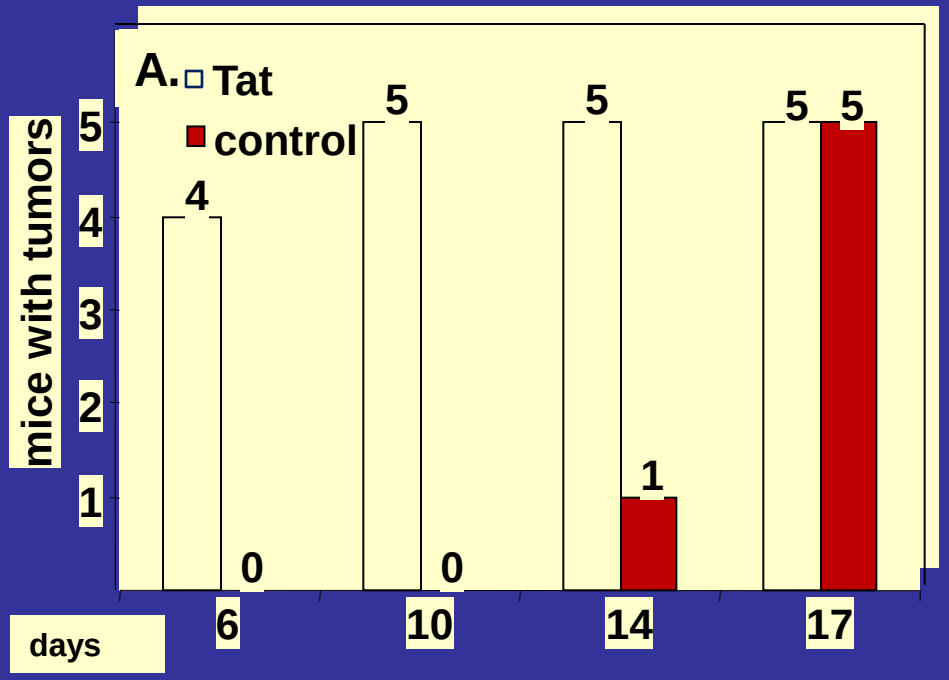
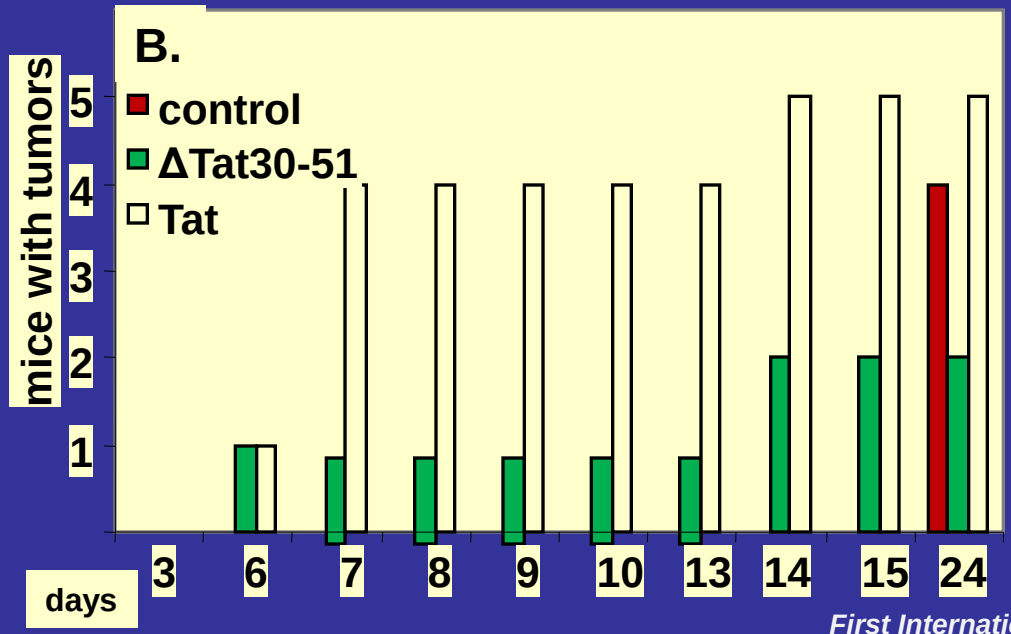


Expression of Tat in normal mouse cells accelerates tumor development from GR3 cells inoculated into nude mice.

A. EC and GR3 cells were mixed.
B. EC and GR3 cells were not mixed; injected into opposite sides of mice



Tat mutant is not effective

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Merkel cell carcinoma virus

- **Merkel cell carcinoma (MCC) is an aggressive skin cancer**
- **MCV is a polyoma virus**
- **Role and mechanism not clear**
- **Clonally integrated MCV found in 80% of MCC; 20% are negative**
- **most MCV isolates from tumors are non-infectious**
- **Most infected people do not develop MCC**
- **UV light (sunlight), HIV may be co-factors for MCC**

Hepatitis B Virus

-- Hepadna virus, DNA virus with 3- kbp circular DNA partially single stranded genome, replicates via reverse transcriptase and an RNA intermediate

-- Thought to cause the majority of hepatocellular carcinomas (HCC)

---Mechanism not clear, probably multifactorial

- ---Usually preceded by cirrhosis

--Damage and inflammation occur from immune attack

-- X protein (HbX) has plieotropic affect on cell activities (cell cycle arrest, apoptosis, etc)

-- Integration into host cell DNA with deletions in pre-S protein may predispose to HCC, and could involve insertional mutagenesis.

What, then, are the most current ideas on mechanism of HCC?

Liver damage from CTLs? X protein? Insertional mutagenesis?

A key question: do all neoplastic cells have HBV sequences? If so are they clonal? To me this is not at all clear.

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Hepatitis C Virus

-HCV is a flavivirus, a positive single stranded RNA virus of 9600 bases with a single ORF, and replicates via an RNA-dependent RNA polymerase, which is so error-prone that HCV is a quasispecies

-Chronic infection can lead to fibrosis, then cirrhosis, then hepatocellular carcinoma (HCC)

-HCC pathogenesic mechanism is not at all clear

-Cofactors include cirrhosis, alcoholism

-Does immune collateral damage contribute to HCC?

-Does liver damage/regeneration contribute to HCC?

-Does HCV make any direct contribution to HCC? Is HCV in the tumor cells? Could there be a hit and run effect? So very little is clear on key questions.

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Hepatitis C Virus

- **Does HCV causes lymphoma?**
- **If so, HOW?**