Stem Cell & Leukaemia

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Outline

• Stem Cell?
  – What is stem cells
  – Types/Sources of stem cells
  – Uses of Stem Cells
  – Stem Tells in Organ transplantation

• What leukaemia?
  – Classification
  – Causes
  – Acute vs Chronic Leukaemias
  – Clinical Features
  – Laboratory Investigation
  – Treatment
  – Prognosis

• References
What is Stem Cell?
Adult /Somatic Stem cells
Two Types of Stem Cells

• Specialised (Adult or somatic)/committed

• Unspecialised (embryonic)/uncommitted
Hemopoietic GF-Cell Differentiation

SCF= Stem Cell Factor
Tpo= Thrombopoietin
Epo= Erythropoietin
IL= Interleukin

GM-CSF= Granulocyte Macrophage-CSF
M-CSF= Macrophage-CSF
G-CSF= Granulocyte-CSF

SDF-1= Stromal cell-derived factor-1
FLT-3 ligand= FMS-like tyrosine kinase 3 ligand

TNF-a = Tumour Necrosis Factor alpha
TGFβ = Transforming GF beta
Source of Stem cells

1. Bone Marrow

2. Peripheral Blood (few)

3. Umbilical cord Blood

• A stem cell transplant is sometimes called a bone marrow transplant.

• *The terms stem cell transplant, infusion, rescue, engraftment, or support may be used interchangeably and essentially have the same meaning.
Stem Cells -BM

• Hemopoietic stem cells

• Myelocytopenesis, erythropoiesis, megakaryocytes

• Erythropoietin, Thrombopoietin, G-CSF, cytokines, interleukins
Adult Stem Cells

- Specialised/Non-functional
- Serve as internal repair system repairing damaged tissues
- Hemopoietic, neural, skin, mesenchymal, organs etc
- Usually give rise to one type of specialised cells on differentiation
- Can not easily be cultured to appropriate quantities required
- Difficult to isolate from tissues
- Less chance of rejection

Embryonic SC

- Pleuripotential/Unspecialised cells
- Potential to develop into other tissue or cell types/repair damage
- Unlimited ability to divide
- Can be induced with appropriate stimulus to become a specialised tissues
- Abundant/embryo tissue culture/IVF
- Laboratory culture techniques well developed to acquire large quantities
• 1998- 1\(^{st}\) ability to grow human embryonic stem cells through IVF technology

• Trans-differentiation (i.e. hemopoietic SC differentiating to muscle cells reported) reported in animals, humans debatable

• Reprogramming of adults SC shown (e.g. reprogramming of other pancreatic cells to beta cells to secrete insulin)
Uses/Importance of Stem Cells

- Cure Disease
- Stem cell therapy
- Nerve repair (paraplegia)
- Kidney Repair (Kidney Renal Failure)
- Replace organ transplant
- Drug testing experiments
Types of Stem cell Transplant

• **autologous** - stem cells harvested from self

• **allogeneic** - stem cells harvested from donor (following reduced for full intensity therapy)

  **syngeneic** - stem cells harvested from identical twin

  **cord blood** - stem cells from saved cord blood, from self or donor
3 Phases of stem cells Transplant

- Phase 1. Identification of Potential Donor
  - Infection screen, matching/HLA typing

- Phase 2. Conditioning phase/Myeloablation –
  - Uses high dose therapies to eradicate the disease/kill cancer cells
  - Destroy immune system
  - Takes 5-10 days

- Engraftment Phase –
  - Infuse stem cells from a donor reconstitute the immune system.
  - Sometimes purging techniques are used to clean the stem cells of residual tumor cells prior to engraftment, or shortly after.

  - 2-4 weeks 1st evidence of “engrafting” or beginning to grow.
  Once “engrafted” and pt’s condition is stable, patients discharged from the hospital.
• **Potential Advantages of Allo Transplants**
  – Tumor free graft
  – Undamaged Stem Cells
  – Avoidance of MDS/secondary AML
  – Graft versus lymphoma effect

• **Potential Problems with Allo Transplants**
  – Lack of suitable donors
  – High Treatment Related Mortality
    Regimen related toxicity
  – Infection
  – Graft versus Host Disease
Success of transplanted Stem Cells depends on its ability to:

• Proliferate extensively and generate sufficient quantities of tissue

• Differentiate into the desired cell type(s)

• Survive in the recipient after transplant.

• Integrate into the surrounding tissue after transplant.

• Function appropriately for the duration of the recipient's life

• Avoid harming the recipient
Future Research

• Tissue locations of adult SC?
• Origin of adult SC (embryonic?)?
• Normal Maintenance/Survival of ASC?
• Can it trans-differentiate?
• Control mechanism for differentiation?
What is Leukaemia

• Definition: Cancer of white blood cells resulting from disordered normal regulation of haemopoietic stem cell proliferation
Classification of Leukaemia

• Acute verses Chronic

• Lymphoid verses myeloid

  – Acute Myeloid Leukaemia
  – Acute Lymphocytic Leukaemia
  – Chronic Myeloid Leukaemia
  – Chronic Lymphocytic Leukaemia
Cause

• Unknown (majority)

• Known causes: Radiation/toxins/chemotherapy agents/myelodysplasia/chrom translocation)
  
  – Toxins (benzene),
  – chemotherapeutic (cyclophosphamide, melphalan, other alkylating agents, and etoposide)

  – May develop from Myelodysplastic syndrome (asso. chrom 5 & 7 abnormalities)

• Subtype, acute promyelocytic leukemia (APL) -translocation t(15;17), → fusion gene PML-RAR which interacts with the retinoic acid receptor to produce a block in differentiation that can be overcome with pharmacologic doses of retinoic acid
General Considerations

- Malignancy of the hematopoietic progenitor cell.

- Proliferate in an **uncontrolled** fashion and replace **normal bone** marrow elements.

- **ALL** Accounts for 80% of Leukaemia in childhood (peak 3-7 years)

- **AML** is primarily an adult disease (median age: 60 years and increases with aging)
General Consideration

• Symptoms result from replacement of normal BM cells by the malignant cell or less commonly from organ infiltration (skin, gastrointestinal tract, meninges).

• Acute leukemia is potentially curable with combination chemotherapy.
Clinical Findings
Symptoms and Signs

• Sick only for days or weeks.

• **Infections:** (neutropenia) <500/mcL; (Gram negatives or (Candida, Aspergillus). Common presentations include cellulitis, pneumonia, and peri-rectal infections;
Thrombocytopenia

**Bleeding:** (low PLT) - skin and mucosal surfaces, with gingival bleeding, epistaxis, or menorrhagia. Less commonly, DIC (APL & monocytic leukaemia).
Acute myelomonocytic leukaemia
Gingival hypertrophy & hemorrhages
Mucosal hematoma-AML
• **Anaemia**: loss of appetite, weak, tired, breathless

• **Other**: gum hypertrophy and bone and joint pain.

• **headache, confusion, and dyspnea** (hyperleukocytosis, with markedly elevated circulating blast count (usually > 200,000 cells/mcL)---->Impaired circulation
On examination

- Pallor (anemia)
- Purpura and petechiae;
- +/- signs of infection (fever/chills/rigor).
- +/- Stomatitis and gum hypertrophy (monocytic leukemia) or rectal fissures.
- +/- hepatosplenomegaly or Lymphadenopathy
- +/- Bone tenderness (sternum, tibia, and femur)
Laboratory Findings

• Pancytopenia & Leukocytosis >200,000/mcl & blast cells

• Peripheral Blood -circulating blasts- (10% cases absent)

• BM –hypercellular (>20% blast cells)

• Other abnormalities: +/-hyperuricemia, DIC, prolonged PT, increased FDP/D-dimer
• Metabolic abnormalities:
  – hyponatremia,
  – Hypokalemia & very high LDH,
  – hyperuricemia, and (rarely) lactic acidosis.

• Hyperkalemia & hypoglycemia
Bone Marrow-AML

hypercellular (>20% blast cells)
Auer Rods, (eosinophilic needle-like inclusion in the cytoplasm -- pathognomonic of AML)
Auer Rods
Acute lymphocytic leukemia (ALL)

Age: 2-10 yrs: 2nd peak: elderly Pts. Only 50% with lymphoblasts in their peripheral blood. (lymphoblasts and a neutrophil.)
Mediastinal Mass (CXR)

Especially T-cell type
• Meningeal leukemia (blasts in CSF in ~5% of cases; more common in monocytic types of AML.
Acute Myeloid Leukaemia

Myeloblast.
Blood Film

- F/60 yr
- PC: gross splenomegaly.
- FBE: Hb = 7.5 g/dl. platelets 14x10^9/l, & WCC 900x10^9/l.
Specialised Tests

• **AML:**
  – **Histochemical staining:** confirms myeloid nature of the cells (peroxidase stain). Monocytic lineage (butyrate esterase)

• **ALL**
  – no morphologic or histochemical evidence of myeloid or monocytic lineage
  – Confirmed by surface markers (**flow cytometry**) – characteristics of primitive lymphoid cells, -----terminal deoxynucleotidyl transferase (TdT) is present in 95% of cases of ALL.
  – **Monoclonal antibodies:** CD19, CD10, CD2, CD5, and CD7.
WHO classification of AML

- Based on morphology and histochemistry (incorporates cytogenetic, molecular and immunophenotypic info)

- Acute undifferentiated leukemia (M0),
- Acute myeloblastic leukemia (M1),
- Acute myeloblastic leukemia with differentiation (M2),
- Acute promyelocytic Leukaemia (M3),
- Acute myelomonocytic leukemia (M4),
- Acute monoblastic leukemia (M5),
- erythroleukemia (M6), and megakaryoblastic leukemia (M7)
WHO classification of ALL

• classified by immunologic phenotype as follows:
  – common,
  – early B lineage,
  – and T cell.
Acute Leukemia
Essentials of Diagnosis

• Short duration of symptoms, including fatigue, fever, and bleeding.

• Pancytopenia.

• More than 20% blasts in the bone marrow.

• Blasts in peripheral blood in 90% of patients.

• Classify as AML or ALL.
Differential Diagnosis

• **AML**: Myeloproliferative disorders, CML and myelodysplastic syndromes.

• **ALL**: CLL, lymphomas, and hairy cell leukemia.

• It may also be confused with the atypical lymphocytosis of mononucleosis (EBV) and pertussis (Bordetella pertussis)
Treatment

• Objective: effecting a cure, i.e.,

• Obtain complete remission, defined as normal peripheral blood with resolution of cytopenias, normal bone marrow with no excess blasts, and normal clinical status.

• The type of initial chemotherapy depends on the subtype of leukemia
Prognosis

• AML
  – Chemotherapy: - Cytarabine & daunorubicin
  – 70–80% of adults with AML under age 60 years achieve complete remission.
  – Prognosis better for younger than older person
  – Transplant superior to Chemotherapy alone
• ALL
  – Vincristine & prednisolone & daunorubicin
  – Prognosis: children better (80%) than adults
Cytogenetic studies are the most powerful prognostic factors

AML:
- Good: t(8;21), t(15;17), and inv(16)(p13;q22).
- Bad: monosomy 5 and 7 and complex abnormalities

ALL:
- Good: hyperdiploid states.
- Bad: Philadelphia chromosomes t(9;22) and t(4;11).
CHRONIC LEUKAEMIA

- CML - myeloid cell origin
- CLL - lymphoid cell origin
Chronic Myeloid Leukaemia

• Is a myeloproliferative disease
  – (others are Essential Thrombocytopenia, Myelofibrosis, Polycythemia Rubra Vera

  – ----→ Overproduction of myeloid cells.

• Early phases; ----myeloid cells retain the capacity for differentiation, & Normal bone marrow function is retained.

• May remain stable for many years & transform to a more overtly malignant disease.

• CML [t (9 & 22)] -Philadelphia chromosome, a reciprocal translocation between the long arms of chromosomes 9 and 22.
• A large portion of 22q is translocated to 9q, and a smaller piece of 9q is moved to 22q.

• Activation of proto-oncogenes (abl/bcr) – tyrosine kinase

• Early CML ("chronic phase") does not behave like a malignant disease.

• Over time CML/without treatment it transforms to AML
Philadelphia Chromosome

Reciprocal translocation

Proto-oncogene

Tyrosine kinase activity
CML (Peripheral Blood Smear, 50 x.).

leucocytosis:
Elevated neutrophils, bands, metamyelocytes, myelocytes, and promyelocytes & basophil eosinophilia

PLT count: normal

(Courtesy of L Damon)
Clinical Findings
Symptoms and Signs

• Age: middle age (median age: 55 years).
• Non-specific symptoms (hypermetabolic-increased WBC)
  – Fatigue & night sweats,
  – low-grade fever,
  – weight loss.

• Splenomegaly--------abdominal fullness/pain

• Incidental Discovery------Elevated WBC

• Rarely: leukostasis with blurred vision, respiratory distress, or priapism.

• WBC usually >500,000/mcL.
On examination

- Severe splenomegaly (like TSS)
- +/- Sternal tenderness – sign of marrow overexpansion.
- Incidental leucocytosis-asymptomatic
- Acceleration of the disease is often associated with fever in the absence of infection, bone pain, and splenomegaly
Laboratory Findings

- Elevated WBC count $>150,000$/mcL,

- Peripheral Blood Film

- The myeloid series is Left –shifted (promyelocytes, myelocytes, blasts)

- Blasts $< 5\%$.

- +/-Basophilia and eosinophilia of granulocytes

- RBC-normal

- Platelet: normal or elevated.
CML. (Bone marrow aspirate, 20 x.) Intense myeloid activity. Dramatic increase in the M:E ratio

Markedly raised megakaryocytes and the number of more immature forms. (Courtesy of L Damon.)
CML. (Bone marrow core biopsy, 20 x.) hypercellularity with the absence of bone marrow fat; extreme myeloid activity and an increased number of megakaryocytes, consistent with this form of chronic leukemia. (Courtesy of L Damon.)
• PCR: $bcr/abl$ gene (supplanted cytogenetics)

• BM examination: is not necessary for diagnosis,

• BUT useful for prognosis and for detecting additional chromosomal abnormalities in addition to the Philadelphia chromosome.

• Progression to AML with Blast transformation: blasts $>30\%$ of BM cells.
Essentials of Diagnosis

• Strikingly elevated white blood count.

• Markedly left-shifted myeloid series but with a low percentage of promyelocytes and blasts.

• Presence of Philadelphia chromosome or bcr/abl gene.
Differential Diagnosis

• Early CML vs reactive leukocytosis associated with infection.

• In such cases, the white blood count is usually less than 50,000/mcL, splenomegaly is absent, and the $bcr/abl$ gene is not present.

• CML vs other myeloproliferative disease
  
  – Normal haematocrit & RBC morphology, and nucleated red blood cells are rare or absent.

  – Definitive diagnosis is made by finding the $bcr/abl$ gene.
Treatment

• Not urgent since the majority of circulating cells are mature myeloid cells that are smaller and more deformable than primitive leukemic blasts.

• **Imatinib mesylate**,  
  – inhibits the tyrosine kinase activity of the *bcr/abl* oncogene.

  – It is well tolerated and results in nearly universal (98%) hematologic control of chronic phase disease.

  – Common SE: nausea, periorbital swelling, edema, rash, and myalgia, but most of these are modest.
Bone Marrow Transplant:

- The only proven curative therapy for CML is allogeneic BM transplantation.

- The best results (80% cure rate) are obtained in patients who are under 40 years of age and transplanted within 1 year after diagnosis from HLA-matched siblings.
Course & Prognosis

- In the past, median survival was 3–4 years.

- With era of **imatinib therapy**, and with the recent development of new molecular targeted agents, more than 80% of patients remain alive and in remission at 4 years.

- It is clear that the prognosis of CML has been dramatically altered by new therapies.

- Allogenic stem cell transplant: only proven curative option, but it is possible that some fraction of patients may be cured by well-tolerated oral agents.
Chronic Lymphocytic Leukaemia
General Considerations - CLL

- Is a clonal malignancy of B lymphocytes.

- Slowly progressive accumulation of long-lived small lymphocytes.

- Cells are immunoincompetent and respond poorly to antigenic stimulation.

- Cells infiltrate BM and suppress normal activity
• Immunodeficiency --- $\rightarrow$ inadequate antibody production

• Direct tissue infiltration --- $\rightarrow$ organ/tissue damage

• Information about CLL is now evolving rapidly, with new findings in biology and new treatment options
Clinical Findings

Symptoms and Signs

• Age: CLL is a disease of older patients (90% over 50, medians 65)

• Incidental finding of lymphocytosis

• Non-specific symptoms: fatigue or lymphadenopathy

• Examination:
  – Lymphadenopathy: (80%)
  – Hepatosplenomegaly (50%)
  – Anaemia & thrombocytopenia (later)
• Associated autoimmune hemolytic anemia or autoimmune thrombocytopenia (5-10%)

• some subtypes behave more aggressively (prolymphocytic leukemia)

• Isolated lymphadenopathy may transformation in into an aggressive large cell lymphoma *(Richter's syndrome)* -5%
Rai Staging System

• stage 0, lymphocytosis only;

• stage I, lymphocytosis plus lymphadenopathy;

• stage II, organomegaly;

• stage III, anemia; and

• stage IV, thrombocytopenia.
Laboratory Findings

• Lymphocytosis. (WBC count >20,000/mcL)

• Usually 75–98% of the circulating cells are lymphocytes.

• Lymphocytes:
  – small and mature, with condensed nuclear chromatin,
  – morphologically indistinguishable from normal small lymphocytes,
  – but smaller numbers of larger and activated lymphocytes may be seen.
• Hematocrit and platelet count are usually normal at presentation.

• BM Infiltration by small lymphocytes

• Immunophenotype --positive CD19 (B-cells) and CD5 (T-cell marker)
• Hypogammaglobulinemia (50%)- common as diseases advances.

• +/-IgM paraprotein is present in the serum.
Chronic Lymphocytic Leukaemia

Mature lymphocytes are increased markedly in number. They are indicative of CLL, a disease most often seen in older adults. This disease responds poorly to treatment, but it is indolent
Differential Diagnosis

- Viral infections
- Pertussis
- Waldenström's macroglobulinemia, (IgM myeloma)
- Hairy cell leukemia, or lymphoma
Essentials of Diagnosis

- Most patients asymptomatic at presentation.
- Splenomegaly typical.
- Lymphocytosis > 5000/mcL.
- Mature appearance of lymphocytes.
- Coexpression of CD19, CD5.
Treatment

• Most cases of early indolent CLL require no specific therapy------ just observe

• Indication for early treatment

  – progressive fatigue, symptomatic lymphadenopathy, or anemia or thrombocytopenia. Ie Rai stage II---II/IV disease.

• Treatment of choice: fludarabine + antibody rituximab (6month)

• Others (fludarabine plus cyclophosphamide +/- rituximab
• Chlorambucil, ---old treatment

• Add prednisolone or splenectomy if signs of autoimmune hemolytic anemia or immune thrombocytopenia

• Immunoglobulin treatment if risk of infection is severe.
• Allogeneic transplantation offers potentially curative treatment for patients with CLL, but it should be used only in patients whose

• BM transplant if not responsive to drugs
Prognosis

• New therapies are changing the prognosis of CLL. In the past, median survival was approximately 6 years, and only 25% of patients lived more than 10 years.

• Stage 0- I median survival of 10–15 years

• Stage III or stage IV
  – Previously (median survival <2 years)
  – With fludarabine-based combination therapies, 2-year survival >90% of patients)
References

• www.stemcells.com

• Current Medical Diagnosis & Treatment 2008

• Harrisons Principle of Internal Medicine 17th Edition