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Demystifying the Cluster Differentiation (CD) System and Clinico-pathological Implications

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Abstract

The cluster of differentiation (CD) is a nomenclature system that identifies and classifies antigens found on the cell surface of various immune and non-immune cells. CD markers are cell surface proteins often used to determine the identity of various cell types. Applications such as flow cytometry and immunohistochemistry can enable phenotypic characterisation of cells based on the expression by unique CD markers. CD markers are also a useful tool for studying the differentiation and maturation of leukocytes and lymphocytes subsets. The HLDA (Human Leukocyte Differentiation Antigens) workshop, which started in 1982, developed the CD nomenclature and has maintained the list of CD Markers ever since. The number of CD markers has grown constantly since its discovery and has expanded to other cell types besides leukocytes. Today, there are more than 370 CD clusters described in humans. CD molecules have varied functions, often act as receptors or ligand and some CD proteins play a role in cell signaling, cell adhesion, cell inhibition and cell activation. CD markers are being increasingly used for diagnosis and follow-up of haematological malignancies, autoimmune diseases, immunodeficiencies, monitoring of cancer immunotherapy and in stem cell biology research. Evaluation of CD markers is not only of diagnostic value at disease onset, but also serve as prognostic and predictive markers to contribute to the treatment of disease and predict its relapse.

Key words: CD, classification, targeted therapy, surface marker.

Introduction

The cell surface is the site of many important biological processes, whichare involved in the interaction between the cell and its environment[1]. Cell membrane proteins comprise approximately 30% of total human proteins; and play a key role in various physiological functions and pathological conditions[2]. The Cluster Differentiation (CD) system is a classification system used to identify and categorise cell surface molecules, known as clusters of differentiation[3]. CD antigens were originally defined as being present on cell surface of leucocytes and recognised by specific antibody molecule, but now also include some intracellular molecules and molecules present on cells other than leucocytes. In some cases, CD antigens are expressed only at certain stages of development or under certain conditions. CD antigens are integral in several immune functions including cell activation, cell adhesion, and immune response regulation. They serve as receptors and ligands, regulate cell signalling and participate in adaptive immunity[4]. While using one CD molecule to define populations is uncommon, combining markers has allowed for cell types with very specific definitions within the immune system. CD markers have proven critical for the identification and isolation of leucocytes, lymphocyte subsets, diagnosis and follow-up of haematological malignancies, autoimmune diseases, immunodeficiencies, monitoring of cancer immunotherapy and in stem cell biology research. However, there are important gaps in our knowledge of CD molecule expression profiles with newer discoveries coming up each day. This review gives a brief outline on CD system and provides a rationale for their usefulness in the current era. The overlay of the review will be as described as under:

- The CD Nomenclature
- The CD classification based on functions
- Methods of Identification of CD makers
- Clinicopathological implications
- Summary

The CD nomenclature

The CD nomenclature system was first introduced and established on the 1st Human Leukocyte Differentiation Antigen (HLDA) Workshop held in Paris in 1982[5,6,7]. CD nomenclature has since been universally adopted by the scientific community and is officially approved by the International Union of Immunological Societies and sanctioned by the World Health Organisation[8]. CD antigens are recognised by antibodies. Monoclonal antibodies that have similar patterns of reactivity with various tissues or cell type are assigned to a cluster group. An antigen well

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recognised by group of antibodies can be assigned a cluster of differentiation number or CD number once two distinct monoclonal antibodies have been demonstrated to bind to that molecule[9]. The HLDA Workshops implemented a standard nomenclature for clusters of antibodies that reacted with a specific antigen, providing consistency and uniformity in manuscripts referring to identical molecules[8,10]. In addition to defining the CD nomenclature, these workshops have been instrumental in identifying and determining the expression and function of cell surface molecules[8]. Over time, the data generated by the 10 HLDA workshops have led to the characterisation and formal designation of more than 370 CD unique clusters and subclusters. The number of CD markers has grown constantly since its discovery and today there are more than 371 CD clusters described in humans[11,12]. Cluster Differentiation (CD) + Number uses the prefix "CD" followed by a number (e.g., CD3, CD20). Each number represents a specific molecule, with some CDs covering a group of closely related family of proteinsor carbohydrates (e.g., CD1a, CD1b, CD1c, and CD1d). A lower case letter following the CD number (e.g., CD1a) indicates several molecules that share a common chain. Other examples are the integrin chains CD11a, CD11b, and CD11c, all of which share CD18 as a common chain to form different dimers[8,12]. In other cases, lower case letters have been used to name different members of the same gene family, as is the case with CD66 (CD66a, CD66b, CD66c, CD66d, CD66e, and CD66f). In the past, an upper case letter was added to some CDs to group related molecules under the same CD number. This was the case for selectins: CD62L (L-selectin), CD62E (E-selectin), and CD62P (P-selectin). Unfortunately, this turned out to be confusing, because sometimes an "L" was added by some researchers to indicate "ligand," such as for CD154, commonly referred to as CD40L. To avoid confusion, the addition of uppercase "L" has been discontinued and should be avoided[8,13]. Provisional indicator "w" is used if the molecule has not been well-characterised, or has only one monoclonal antibody, as in "CDw186"[8]. The CD designations were used to describe the recognised molecules but had to be clarified by attaching the term antigen or molecule to the designation (e.g., CD2 molecule). Currently, "CD2" is generally used to designate the molecule, and "CD2 antibody" is used to designate the antibody that reacts with CD2 antigen[14]. In the context of cell populations, the presence or absence of a CD molecule is often denoted using '+' or '–' symbols. For example, a "CD34+, CD31–" cell denotes a cell expressing CD34 but lacking CD31. Additionally, some cell populations can be classified as hi, mid, or low (alternatively bright, mid, or dim), indicating varying levels of CD expression[15]. Monitoring the presence, absence and expression profiles of different CD antigens can identify, isolate, and immunophenotype cells in immune processes. Fig. 1 illustrates an example of CD antigen on surface and its corresponding antibody reacting with the specific CD antigen.

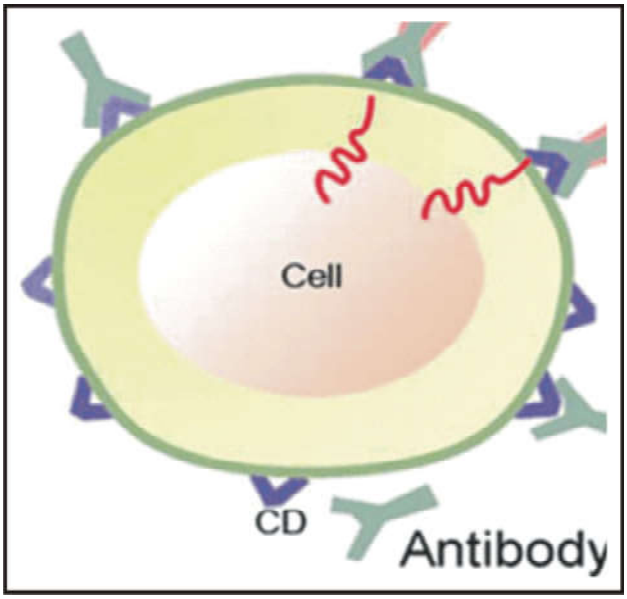

Fig. 1: *Graphical representation of CD antigen on the surface and reactivity by corresponding antibody.*

The CD Classification based on functions

The classification of CD clusters and subclusters is based on the distinct markers on cells that serve as unique identification tags and can readily be distinguished by determining such combination of molecules on their membranes. CD markers have specific functions and can be differently expressed in response to environmental conditions and intracellular genetic changes[16]. Not only the presence of these CD markers but also the absence of expression of CD makers can give a clue to the correct diagnosis. Following descriptions classify CD markers based on their varied functions.

a. Role of CD Markers as cell surface receptors

CD markers can be recognised by specific monoclonal antibodies, generated against the epitopes on the cell surface which serve as cell receptors, facilitating cell recognition and interaction, thereby influencing immune responses, and shaping the intricate dynamics of the immune system. CD markers are found in various immune cell populations like B cells, T-cells, dendritic cells, NK cells, monocytes, macrophages, endothelial cells, epithelial cells, red blood cells, granulocytes, platelets and stem cells[17]. As lymphocytes mature, they express different protein receptors on the cell surface, which can aid in determining the type and maturation stage of the cells being examined.

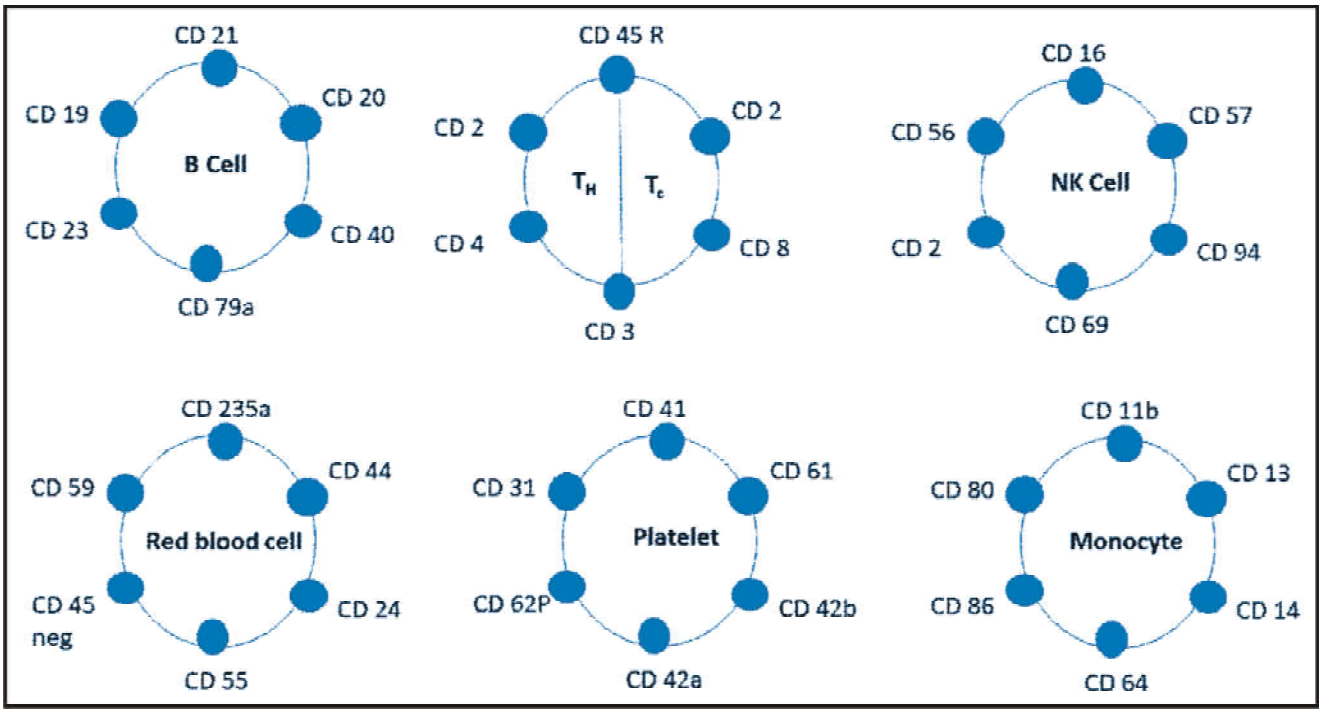

Fig. 2: *Diagrammatic representation of CD antigen distribution on various immune cells.*

CD antigen distribution on various immune cells is illustrated graphically in Fig. 2.

b. Role of CD Markers in other functions

The CD markers play a significant role in signal transduction, cell adhesion, cell migration, cell activation, cell to cell interaction, cell inhibition and in adaptive immunity[18]. Depending on the functional properties, various CD markers have been identified which play a varied role in physiological functions such as:

CD antigens in signal transduction: When a CD antigen activates its receptor, the signal is carried into the cell by means of a second messenger. Example, the marker CD47 is found to have anti-phagocytic signals to macrophages and inhibit natural killer (NK) cells. This enabled researchers to apply CD47 as a potential target to attenuate immune rejection[19,20].

CD antigens in cell adhesion, migration, activation, cell to cell interaction and cell inhibition: Some CD antigens act as cell-cell or cell-matrix adhesion molecules, by which cells form contacts with each other or with their substratum through specialised protein complexes. This intricate mechanism plays a pivotal role in shaping tissue structure during morphogenesis and maintaining tissue cohesion in post-developmental life. CD antigens play a significant role in cell migration and guide immune cells to specific locations in the body. For instance, CD44 aids lymphocyte homing to lymph nodes, and selectins interact with CD15 and CD62L, facilitating leukocyte recruitment to inflammatory sites[21]. CD antigens are also critical to mediating cell interactions. These surface molecules on immune cells, like T-cells and antigen-presenting cells, enable recognition and communication between cells. For example, CD4 and CD8 interact with T-cell receptor (TCR) and major histocompatibility complexes (MHC), playing a crucial role in T-cell activation[22,23]. CD antigens also play a key role in regulating cell inhibition. For instance, programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) are CD antigens that, when bound by their ligands, inhibit T-cell activation[24]. CD antigens are used to identify the maturation or activation stage of various cells, including T-cells, B-cells, and other immune cells. For example, in dendritic cells, the most important CD markers of activation are CD80, CD86 and CD83[25].

CD markers in adaptive immunity: The adaptive immune or specific immune response consists of antibody responses and cell-mediated responses, which are carried out by different lymphocyte cells, B-cells, T-cells, NK cells, dendritic cells and monocytes[26]. CD molecules are essential markers for the identification and isolation of immune cells. As lymphocytes mature, they express different protein receptors on the cell surface which helps in determining the type and maturation stage of lymphocytes[18], e.g., CD 45 is the pan leucocyte marker, while CD3 surface antigens

form part of the T-cell receptor complex for antigens. CD3 is expressed exclusively by mature lymphocytes of the T-cell lineage. CD4 and CD8 are subtypes of CD3+ T lymphoid cells and used as markers for the helper and cytotoxic T lymphoid cells, respectively. CD 19 and CD20 are seen exclusively on lymphocytes of the B-cell lineage. In addition, CD 25 expression on T lymphoid cells can serve as markers of activation.

Table I highlights few common CD antigens related to various functions and their expression on various cells.

Methods of identification of CD markers

CD antigens are widely used as cell markers in immunophenotyping thereby allowing the identification of the presence or absence of cell markers as well as to quantify proportions of specific cell populations and lymphocyte subsets. Immunophenotyping is a powerful tool that uses assays like flow cytometry (FCM) or immunohistochemistry (IHC) to gain insights into the composition and dynamics of cell populations and can be performed to detect and quantify CD markers. These techniques involve identifying cell types in heterogeneous cell populations by using different antibodies that target various CD markers. Different combinations of antibodies can be used to identify different groups and sub-groups of cells. By applying CD markers in immunophenotyping techniques, scientists and clinicians can discern specific cell types within complex immune responses, facilitating disease diagnosis, treatment development, and immunological research. The following immuno-phenotyping techniques are commonly used for the identification of CD markers:

Flow cytometry is a common technique to analyse the expression of CD markers on single cells in fluidic medium[27]. FCM involves obtaining a sample of cells, such as blood, bone marrow, or tissues, and incubating them with a panel of fluorescently labelled antibodies that recognise specific cell surface markers[27]. The antibodies can target various CD markers, the labelled cells are then passed through a flow cytometer, which detects and measures the fluorescence emitted by each individual cell, providing quantitative data about the expression levels of different markers. FCM is a key technique for the immunophenotypic diagnosis of acute leukaemia's and chronic lymphoproliferative disorders, with the ability to provide data on simultaneous evaluation of multiple proteins in hundred thousand to millions of single cells[28]. Most CD antigens are expressed at varying levels by many different cell types. Rather than the exclusive expression of a single CD antigen with a particular cell type, it is the peculiar constellation of surface antigens expressed by a given cell that helps assign it to a particular lineage or sub lineage of cells. The resolution of cell subpopulations usually requires two or more colour FCM analysis. Advantage of FCM is that it allows for the simultaneous detection of several markers on a single cell at the same time. A modern 8 - 10 colour FCM can simultaneously measure the expression of 8 - 10 CD markers on single cell, most advanced FCM can analyse upto 18 CD markers also[29]. For example, CD3 can be used as a general marker for T-cells followed by other CD markers to identify regulatory T-cells (CD4 and CD25), T helper cells (CD4), and cytotoxic T-cells (CD8). In case of lymphoid neoplasms, for example if abnormal lymphoid cells are CD 5 positive and CD23 negative on FCM, the diagnosis favours mantle cell lymphoma and not chronic lymphocytic leukaemia

Table I: Showing few common CD antigens related to various functions and their expression on different cells.

Function of CD markers	B Lymphocyte	T lymphocyte	Dendritic cell	Stem cells	Monocyte	Granulocytes	Endothelial cells	Epithelial cell
Signal Transduction	CD79a, CD73, CD53, CD122, CD77, CD69	CD4, CD21, CD5, CD8, CD38, CD55	CD18, CD86, CD33, CD37, CD40, CD150	CD19, CD21, CD55, CD200, CD22, CD135	CD14, CD206	–	–	–
Cell Adhesion	CD22, CD11c, CD35, CD39, CD44, CD50	CD4,CD6, CD31, CD47, CD99, CD84	CD18, CD48, CD33, CD53, CD191, CD106	CD9, CD81, CD22, CD99, CD324, CD48	CD9, CD11c, CD54, CD22, CD36, CD62L	CD43, CD66b, CD99, CD33, CD58, CD35	CD9, CD50, CD34, CD111, CD239, CD225	CD26, CD40, CD44, CD118, CD54, CD113
Cell Migration	CD11a, CD18, CD31, CD44,CD53,CD97	CD9, CD44, CD54, CD97,CD177,CD304	CD1c, CD5, CD11c, CD14,CD47	–	CD44, CD53, CD99, CD302, CD209, CD321	CD16b, CD29, CD53, CD44, CD97, CD99	CD54,CD106,CD225, CD209, CD228, CD309	CD66a, CD66c, CD228, CD318, CD332, CD362
Cell Interaction	CD138, CD22, CD167b, CD326, CD360	CD1d, CD4, CD7, CD28, CD27, CD278	CD37, CD40, CD47, CD80, CD170, CD146	–	CD62L, CD11c, CD18, HLADR	–	–	–
Cell Activation	CD18, CD33,CD36, CD5, CD30, CD46	CD2, CD9, CD4, CD6, CD28, CD43	CD11c, CD19, CD23, CD37, CD38, CD80	–	CD11c, CD18	CD43, CD55, CD66a, CD99, CD261, CD270	–	–
Cell Inhibition	CD46, CD80, CD86, CD264, CD279	CD1d, CD28, CD59, CD161, CD225, CD273	CD2, CD28, CD40, CD137, CD152	–	CD33, CD118, CD172a, CD300f	–	–	–
Adaptive Immunity	CD19, CD20, CD22, CD35, CD95, CD102	CD4, CD8, CD3, CD55, CD59, CD95	CD22, CD33, CD170, CD320	–	CD5, CD35, CD54, CD64, CD95	CD16a, CD35, CD59, CD281, CD305, CD178	–	–

(CLL), though by morphological diagnosis, they have similar appearance. Not only the presence or absence but the intensity of expression of CD markers can also play a significant role leading to correct diagnosis in certain conditions like loss or reduced expression of CD10 can be a dysplastic event in neutrophils in myelodys plastic syndromes. In a case with morphological diagnosis as leukaemia, by FCM when the blast population express myeloid antigens like CD 13, CD33, CD117, the diagnosis favours acute myeloid leukaemia (AML). Reactivity of the most common antibodies (CD Markers) used in FCM of haematolymphoiddis ordersis as depicted in Table II.

Table II: Depicting CD markers used in flow cytometry for Haematolymphoid disorders.

Cell type	CD marker
All leucocytes	CD45+
T lymphocyte	CD45+,CD2+, CD3+, CD4+, CD8+, CD5+,CD7+
T Helper lymphocyte	CD45+, CD3+, CD4+, CD8-
T cytotoxic lymphocyte	CD45+, CD3+, CD8+, CD4-
Regulatory T-cells (Treg)	CD4, CD25, FOXP3 (a transcription factor)
B Lymphocyte	CD45+, CD19+, CD20+, CD24+, CD38, CD22
NK cells	CD16+, CD56+, CD3-, CD31, CD30, CD38
Red blood cells	CD235,
Monocytes	CD4, CD45+, CD14+, CD114+, CD11a, CD11b, CD16+
PNH markers	CD 55, CD59, CD157 , CD64 ,CD24, CD15 ,CD16
Granulocytes	CD45+, CD11b, CD15+, CD24+, CD114+, CD182+
Myeloid Cells	CD13+, CD33+
Stem cells	CD34 +, CD31-, CD117+
Plasma cells	CD38+,CD138+,CD81+,CD27+

Immunohistochemistry (IHC) is a powerful technique that exploits the specific binding between an antibody and antigen to detect and localise specific antigens in cells and tissue[30]. Many lesions show overlapping features on morphological appearance and is not always sufficient to subcategorise the cancer. IHC by identifying various CD antigens in solid tissues can play an important role in the differential diagnosis of the diagnostically challenging lesions on morphology. IHC can be performed conveniently on formalin fixed paraffin embedded (FFPE) tissue[31,32] and by automated methods for high volume processing with reproducibility[33]. IHC is frequently utilised to determine the CD antigens in solid tissues and bone marrow biopsy sections thereby assisting in the diagnosis and classification of neoplasms, determining a metastatic tumour's site of origin and detection of tiny foci of tumour cells inconspicuous on routine haematoxylin and eosin (H&E) staining[34]. Furthermore, it is increasingly being used to provide predictive and prognostic information as well[35].Interpretation of CD antigens in tissues is based on cellular distribution of antibody staining (i.e., membranous, cytoplasmic, nuclear), proportion of positively stained cells, staining intensity and cut-off levels. The use of IHC for determining CD antigens has recently further expanded to assess predictive and prognostic biomarkers in many malignancies including those of the breast, gastrointestinal tract, lung, haematolymphoid and central nervous systems[36]. Table III depicts CD markers in various solid tumours.

Table III: Highlights utility of CD markers as diagnostic and prognostic markers in solid cancers.

Type of cancer	Commonly identified CD Markers	Prognostic CD markers
Breast	CD44, CD24, CD133, CD14, CD200, CD4, CD8, CD4	CD44, CD133, CD14
Colorectal	CD66, CD110, CD133, CD44, CD2, CD89, CD200	CD110, CD133, CD44, CD200
Lung	CD117, CD176, CD166, CD88, CD103, CD66	CD88, CD103
Liver	CD133, CD44, CD90, CD105, CD34, CD151, CD206, CD68	CD151, CD68, CD206

Clinico-Pathologic Implications of CD markers

1. Cancer Diagnosis and Prognosis:

Haematological Malignancies: CD markers are crucial for diagnosing and classifying leukaemias and lymphomas as a specific set of CD markers are expressed on these cells depending on the stage and pathway of differentiation[16]. Abnormal expression of CD markers in bone marrow and peripheral blood is used as the first diagnostic strategy and is also followed to monitor the clinical course of leukaemia/ lymphomas[37]. For example, CD19 and CD20 are markers for B-cells and are used to diagnose B-cell lymphomas/leukaemia. CD34 serves as a marker for hematopoietic stem cells (HSC) and is frequently used to identify immature or undifferentiated cells in leukaemia, particularly acute leukaemia's. Thus, positivity of CD34 differentiates between blasts in leukaemiavs mature lymphomas. Depending upon the expression of various B cell markers, distinguishing being various lymphomas is also possible Similarly, T markers expression like CD2, CD3, CD4, CD8 on leukaemia/lymphoma cells can determine the T-cell origin. In cases of diagnostic dilemma between similar looking blasts on morphological examination, expression of myeloid markers like CD13, CD33, CD117 can determine AML versus acute lymphoblastic leukaemia (ALL). CD15 and CD30 are associated with Reed-Sternberg cells in Hodgkin lymphoma (HL), thereby leading to correct tissue

diagnosis. CD138 (Syndecan-1) serves as a marker for plasma cells and is used in the diagnosis of multiple myeloma, a cancer of plasma cells.

Prognostic Indicators: CD markersare widely used as both prognostic and predictive markers in immunology and oncology/haematology[38] and provide information about the aggressiveness of the disease and predict patient outcomes. CD38 expression is typically associated with increasing proliferation and survival of malignant B-cells and has been recognised as a poor prognostic marker in CLL[39,40]. CD38 positivity in CLL is associated with aaggressive clinical course compared to CD 38 negative cases. Detection of the changes in CD markers' expression in leukaemia also contributes to the prognosis of these disorders. CD3, CD4, and CD8 are markers used to evaluate the presence and composition of T lymphocytes in the tumour microenvironment, which can have prognostic significance in various cancers[41]. CD163 and CD68 are markers of macrophages and are indicated as a prognostic marker in classical HL with the highest tumour associated macrophages having reduced disease-free survival and overall survival[42,43].

Solid Malignancies: Expression ofCD markers in solid tumours can be studied by IHC on tissue sections or as circulating tumor cells in blood sample by utilising FCM. Identification of CD markers on a specific tumor leads to early detection and also serves as a prognostic factor for monitoring the progression of solid tumors[44]. More recently, CD markers have also been used in detecting potential cancer stem cells[45]. CD44 is linked to cancer stem cells in multiple cancer types, including breast, colon, and pancreatic cancer[46] while CD326 (EpCAM) is employed as a marker in the detection and characterisation of certain epithelial cancers, including breast, colorectal, and ovarian cancers[47]. CD99 is expressed in various types of tumours, including Ewing's sarcoma, small round cell tumors, and some soft tissue sarcomas. CD133, as a putative stem cell marker, is associated with more advanced stages of Wilms and neuroblastoma (NB) tumors; therefore, this molecule can be a potential clinical prognostic marker in children suffering from NB or Wilms tumour[48]. Monitoring of tumor progression through CDs markers expressed on circulating tumour cells could be a new diagnostic and prognostic factor in the future. Table III depicts few CD markers identified in solid tumours serving as diagnostic and prognostic markers.

2. Immunotherapy and Targeted Therapies

CD markers are used to develop targeted therapies. Advances in genetic technology have led to a growing number of approved immunotherapeutic agents. As opposed to older generation chemotherapy which targets fast-replicating cells which can be both cancerous and healthy, these newer generation drugs target only those cells with a specific CD "tag." Immunotherapeutic agents can be monoclonal antibodies which are tagged to drugs or radiation-emitting substances that have the ability to kill cancerous cells expressing the specific CD marker on their surface[49]. For example, CAR-T-cell therapy involves modifying T-cells to express chimeric antigen receptors (CARs) that target specific CD markers on cancer cells, such as CD19 in B-cell malignancies[50]. The predictable expression of CD molecules on various hematologic malignancies has allowed for therapeutic targeting of the malignancy with monoclonal antibodies (MAbs). The most advanced targeted therapy inrecent times is rituximab which has received Food and Drug Administration (FDA) approval and is in wide spread clinical application[51]. Rituximab targets CD20, which is expressed on the majority of B-cells and it has shown great activity in treating CD20-positive B-cell leukemias and lymphomas[52]. CD24, also known as Heat Stable Antigen (HSA), has been extensively studied in the field of immunotherapy in various solid malignancies, and as a novel molecule for targeted drug delivery and imaging[53]. Among the drugs currently approved by FDA for use in immunotherapy is depicted in Table IV.

3. Autoimmune Diseases:

CD markers are routinely used to study the phenotype and functionality of immune cell populations in autoimmunity. FCM is the most useful technique to identify and quantitate CD markers in autoimmunity. CD4+/CD8+ (helper/suppressor) T lymphocyte ratio assessment along with autoantibody detection and HLA-DR+ T lymphocyte measurements have been well studied in autoimmune disorders. Other useful CD biomakers such as TCRα/β, CD-, CD8- double negative T-cells are elevated in autoimmune lymphoproliferative syndromes (ALPS) syndrome. FOXP3+CD25+CD4+ Treg (regulatory T) population is found to be reduced in various autoimmune disorders[54]. Decreased absolute numbers of both CD27+ and CD27– B-cells as well as decreased proportions of IgD+CD27+ memory B-cells are noted in SLE. Thus, combined with other clinical parameters B-cell profiling can help identify potential biomarkers relevant to lupus disease[55]. Cell surface markers such as CD74 can be monitored in multiple sclerosis by FCM not only to assess disease activity and progression, but also to evaluate the clinical efficacy of treatment[56]. In addition, CD19 and CD20 counts are also used as markers to evaluate treatment efficacy of rituximab, a monoclonal antibody directed at CD20+ B-cells.

Table IV: Depicting CD marker as therapeutic targets according to the clinical application of specific monoclonal antibodies.

CD Marker	Disease	Example of theuraptic antibody against CD marker
CD 3	Autoimmune disease, transplant rejection	OKT3 (muromonab), otelixizumab, catumaxomab
CD19	Lymphomas, Acute lymphoblastic leukaemia	Blinatumomab, taplitumomab
CD20	Lymphomas, autoimmune diseases, Immune thrombocytopenia purpura, Chronic lymphocytic leukaemia (CLL)	Rituximab, afutuzumab, ibritumomabtiuxetan, ocaratuzumab, ocrelizumab, ofatumumab, tositumomab
CD30	Hodgkin's lymphoma and anaplastic large cell lymphoma	Brentuximabvedotin
CD33	Acute myeloid leukaemia	Gemtuzumabozogamicin
CD52	Acute leukaemia, SLL/CLL/Peripheral T-cell lymphoma	Campath (Alemtuzumab)
CD75, CD38	Multiple myeloma	Milatuzumab, Daratumumab
CD4	Psoriasis, HIV, autoimmune diseases	Ibalizumab, cedelizumab, clenoliximab, priliximab
CD6	Autoimmune diseases, Sjögren's syndrome	Itolizumab
CD11a	Psoriasis, autoimmune diseases	Efalizumab
CD125	Asthma	Benralizumab
CD340	HER2+ Breast cancer	Herceptin® (trastuzumab)

4. Stem cell enumeration, Transplantation and Graft Monitoring

CD34, is a marker of HSC in bone marrow and blood. Collection and infusion of CD34+ HSC following chemotherapy is critical in bone marrow transplantation[57]. Evaluation of CD34 + HSC harvest adequacy is achieved by CD34 cell counting using FCM as the number of viable CD45+/CD34+ cells will determine the quality of the harvested specimen for bone marrow transplantation. CD markers are also used to monitor and assess graft-versus-host disease (GVHD) in organ transplantation. Certain CD markers help in evaluating the extent of immune response against the transplanted organ. Analysis of peripheral blood CD 8 +T lymphocytes may help to indicate early rejection.

5. Immunodeficiency diseases

Primary immune deficiency disorders (PIDDs) are a group of inherited disorders affecting single or multiple components of the immune system, resulting in increased predisposition to infections and immune dysregulation. A preliminary lymphocyte subset analysis with CD19, CD20 (B-cells), CD 3, CD4, CD8, CD7 (T-cells), NK cells (CD16, CD56) by FCM is the first-line investigation for PIDD. Further subset analysis of B cells and T-cells can be done by utilising CD markers to determine naïve, memory B and T lymphocytes in various immunodeficiency disorders[58].

6. Infectious Diseases

HIV Monitoring:

Many studies have established the utility of CD4+ T-cell count as a critical marker for monitoring HIV infection and progression. The decline in CD4+ T-cells correlates with disease progression and immune system compromise in HIV patients. CD 4 T lymphocyte count is also being used for initial assessment of *in vivo* antiretroviral drug activity and is utilised in determining antiretroviral therapy eligibility and time to initiate therapy[59].

Sepsis: There have been a number of studies looking at the ability of CD64 expression on neutrophils to detect the presence of infection and/or the presence of sepsis. Even quantitative CD64 expression has been shown to correlate with the progression of sepsis to severe sepsis in few studies on critically ill patients[60]. The expression of the integrin CD11b, which enhances the ability of neutrophils to adhere to the endothelium in sites of inflammation, is also increased in bacterial infection, and some have proposed the use of both CD64 and CD11b together to diagnose sepsis[61].

7. CD markers in neuroscience research

CD markers have proven invaluable for the study on neuronal and glial cells thereby providing insights into their functions and interactions[62]. Such identification of specific CD markers is pivotal in investigations related to neural development, neurodegenerative disorders, and brain tumorssuch as glioma. For example, CD56 (NCAM) is associated with neuroendocrine tumours and neural development disorders like autism and schizophrenia[62]. CD133 (Prominin-1) is linked to brain tumour stem cells in glioblastoma, breast cancers and other brain cancers[63]. CD31 (PECAM-1) relates to endothelial cells in the blood-brain barrier and is pertinent in neuroinflammatory conditions.

CD184 (CXCR4) is implicated in HIV-associated neurocognitive disorders (HAND) due to its role in viral entry into the central nervous system.

Summary

Cluster of Differentiation (CD) markers serve as a vital classification system for cell surface molecules not only on immune cells but also on a variety of other cell types. Each CD marker is assigned a unique number that corresponds to a specific cell surface protein or antigen. CD markers have multifaceted roles in immunology, haematology, oncology, neurosciences, stem cell research and immunotherapy. As comprehension of the immune system and cell biology advances, researchers continue to uncover and elucidate the pivotal role of CD markers not only as diagnostic marker but also prognostic and predictive markers in various fields.

References

1. Zola H. Human leukocyte differentiation antigens as therapeutic targets: the CD molecules and CD antibodies. *Expert Opin Biol Ther* 2001; 1 (3): 375-83.
2. Várady G, Cserepes J, Németh A *et al*. Cell surface membrane proteins as personalised biomarkers: where we stand and where we are headed. *Biomark Med* 2013; 7 (5): 803-19.
3. Tian HF, Xing J, Tang XQ *et al*. Cluster of differentiation antigens: essential roles in the identification of teleost fish T lymphocytes. *Mar Life Sci Technol* 2022; 4 (3): 303-16.
4. Cruse JM, Lewis RE, Wang H *et al*. Cluster of Differentiation (CD) Antigens. In: Cruse JM, Lewis RE, editors. *Immunology Guidebook*. Amsterdam: Elsevier, 2004; 47-124.
5. Clark G, Stockinger H, Balderas R *et al*. Nomenclature of CD molecules from the Tenth Human Leucocyte Differentiation Antigen Workshop. *Clin Transl Immunology* 2016; 5 (1).
6. Bernard A, Boumsell L. Human leukocyte differentiation antigens. *Presse Med* 1984; 13 (38): 2311-6.
7. Fiebig H, Behn I, Gruhn R *et al*. Characterisation of a series of monoclonal antibodies against human T cells. *AllergImmunol (Leipz)* 1984; 30 (4): 242-50.
8. Engel P, Boumsell L, Balderas R *et al*. CD Nomenclature 2015: Human Leukocyte Differentiation Antigen Workshops as a Driving Force in Immunology. *J Immunol* 2015; 195 (10): 4555-63.
9. Kumar S, Lütteke T, Schwartz-Albiez R. GlycoCD: a repository for carbohydrate-related CD antigens. *Bioinformatics* 2012; 28: 2553-55.
10. HCDM responsible for HLDA workshop and CD molecules. Human Cell Differentiation Molecules Council. Available from: http://www.hcdm.org/MoleculeInformation/tabid/54/Default.aspx. Accessed 21 Apr 2016.
11. Zola H, Swart B, Banham A *et al*. CD molecules 2006 – human cell differentiation molecules. *J Immunol Methods* 2007; 319 (1-2): 1-5.
12. Zola H, Swart B, Nicholson I *et al*. CD molecules 2005: human cell differentiation molecules. *Blood* 2005; 106 (9): 3123-6.
13. Beare A, Stockinger H, Zola H, Nicholson I. Monoclonal antibodies to human cell surface antigens. *Curr Protoc Immunol* 2008; 80 (1): 4A.1-A.4A.73.
14. Ho IC, Tai TS, Pai SY. GATA3 and the T-cell lineage: essential functions before and after T-helper-2-cell differentiation. *Nat Rev Immunol* 2009; 9 (2): 125-35.
15. Shahrabi S, Ghanavat M, Behzad MM *et al*. CD markers polymorphisms as prognostic biomarkers in hematological malignancies. *Oncol Rev* 2020; 14 (2): 466.
16. Kalina T, Fišer K, Pérez-Andrés M *et al*. CD Maps-Dynamic Profiling of CD1-CD100 Surface Expression on Human Leukocyte and Lymphocyte Subsets. *Front Immunol* 2019; 10: 2434.
17. Deuse T, Hu X. Hypoimmunogenic derivatives of induced pluripotent stem cells evade immune rejection in fully immunocompetent allogeneic recipients. *Nat Biotechnol* 2019; 37 (3): 252-8.
18. Jalkanen S, Saari S, Kalimo H *et al*. Lymphocyte migration into the skin: the role of lymphocyte homing receptor (CD44) and endothelial cell antigen (HECA-452). *J Invest Dermatol* 1990; 94 (6): 786-92.
19. Li QJ, Dinner AR, Qi S *et al*. CD4 enhances T-cell sensitivity to antigen by coordinating Lck accumulation at the immunological synapse. *Nat Immunol* 2004; 5: 791-9.
20. Holler PD, Kranz DM. Quantitative analysis of the contribution of TCR/pepMHC affinity and CD8 to T-cell activation. *Immunity* 2003; 18: 255-64.
21. Buchbinder EI, Desai A. CTLA-4 and PD-1 Pathways: Similarities, Differences, and Implications of Their Inhibition. *Am J Clin Oncol* 2016; 39 (1): 98-106.
22. Schmidt SV, Nino-Castro AC, Schultze JL. Regulatory dendritic cells: there is more than just immune activation. *Front Immunol* 2012; 3: 274.
23. Chaplin DD. Overview of the immune response. *J Allergy ClinImmunol* 2010; 125 (2 Suppl 2).
24. McKinnon KM. Flow Cytometry: An Overview. *Curr ProtocImmunol* 2018; 120: 5.1.1-5.1.11.
25. Pedreira CE, Costa ES, Lecrevisse Q *et al*. Overview of clinical flow cytometry data analysis: Recent advances and future challenges. *Trends Biotechnol* 2013; 31: 415-25.
26. O'Donnell EA, Ernst DN, Hingorani R. Multiparameter flow cytometry: advances in high resolution analysis. *Immune Netw* 2013; 13 (2): 43-54.
27. Bento LC, Correia RP, Mangueira CLP *et al*. The use of flow cytometry in myelodysplastic syndromes: A review. *Front Oncol* 2017; 7: 270.
28. Taylor CR, Shi SR, Barr NJ. Techniques of immunohistochemistry: principles, pitfalls, and standardisation. In: Dabbs DJ, editor. Diagnostic immunohistochemistry: *Theranostic and Genomic Applications*. 3rd ed. Philadelphia: Saunders; 2010.
29. Shi SR, Key ME, Kalra KL. Antigen retrieval in formalin-fixed paraffin-embedded tissues: an enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections. *J Histochem Cytochem* 1991; 39: 741-8.
30. Magaki S, Hojat SA, Wei B *et al*. An introduction to the performance of immunohistochemistry. *Methods Mol Biol* 2019; 1897: 289-98.
31. Wolff AC, Hammond ME, Hicks DG *et al*. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. *Arch Pathol Lab Med* 2014; 138 (2): 241-56.
32. Rizk EM, Gartrell RD, Barker LW *et al*. Prognostic and predictive

immunohistochemistry-based biomarkers in cancer and immunotherapy. *Hematol Oncol Clin North Am* 2019; 33 (2): 291-9.

33. Karai B, Habok M, Remenyi G *et al*. A novel flow cytometric method for enhancing acute promyelocyticleukaemia screening by multidimensional dot-plots. *Ann Hematol* 2019; 98 (7): 1413-20.
34. Parker TL, Strout MP. Chronic lymphocytic leukaemia: prognostic factors and impact on treatment. *Discov Med* 2011; 11 (57): 115-23.
35. Deaglio S, Vaisitti T, Serra S *et al*. CD38 in chronic lymphocytic leukaemia: from bench to bedside? *Mini Rev Med Chem* 2011; 11 (6): 503-7.
36. Deaglio S, Vaisitti T, Zucchetto A *et al*. CD38 as a molecular compass guiding topographical decisions of chronic lymphocytic leukaemia cells. *Semin Cancer Biol* 2010; 20 (6): 416-23.
37. Palomero J, Panisello C, Lozano-Rabella M *et al*. Biomarkers of tumour-reactive CD4+ and CD8+ TILs associate with improved prognosis in endometrial cancer. *J Immunother Cancer* 2022; 10 (12).
38. Werner L, Dreyer JH, Hartmann D *et al*. Tumour-associated macrophages in classical Hodgkin lymphoma: hormetic relationship to outcome. *Sci Rep* 2020; 10 (1): 9410.
39. Xie Y, Yang H, Yang C *et al*. Role and mechanisms of tumour-associated macrophages in hematological malignancies. *Front Oncol* 2022; 12: 933666.
40. Rezaeeyan H, Shahrabi S, McKee TD, Saki N. The expression of CD markers in solid tumours: significance in metastasis and prognostic value. *Histol Histopathol* 2018; 33 (10): 1005-12.
41. Keysar SB, Jimeno A. More than markers: biological significance of cancer stem cell-defining molecules. *Mol Cancer Ther* 2010; 9 (9): 2450-7.
42. Dzobo K, Sinkala M. Cancer stem cell marker CD44 plays multiple key roles in human cancers: immune suppression/evasion, drug resistance, epithelial-mesenchymal transition, and metastasis. *OMICS* 2021; 25 (5): 313-32.
43. Baeuerle PA, Gires O. EpCAM (CD326) finding its role in cancer. *Br J Cancer* 2007; 96 (3): 417-23.
44. Mehrazma M, Madjd Z, Kalantari E *et al*. Expression of stem cell markers CD133 and CD44 in paediatric solid tumours: a study using tissue microarray. *Fetal Paediatr Pathol* 2013; 32 (3): 192-204.
45. Zahavi D, Weiner L. Monoclonal antibodies in cancer therapy. *Antibodies (Basel)* 2020; 9 (3): 34.
46. Sterner RC, Sterner RM. CAR-T cell therapy: current limitations and potential strategies. *Blood Cancer J* 2021; 11 (1): 69.
47. Davis T, Gore-Langton RE, Cheson BD. Current clinical trials of the anti-CD20 monoclonal antibody rituximab. *Oncology (Williston Park)* 2000; 14: 1440-7.
48. Foon KA. Monoclonal antibody therapies for lymphomas. *Cancer J* 2000; 6 (5): 273-8.
49. Yang Y, Zhu G, Yang L, Yang Y. Targeting CD24 as a novel immunotherapy for solid cancers. *Cell Commun Signal* 2023; 21 (1): 312.
50. Cabral-Marques O, Schimke LF, de Oliveira EB Jr *et al*. Flow cytometry contributions for the diagnosis and immunopathological characterisation of primary immunodeficiency diseases with immune dysregulation. *Front Immunol* 2019; 10: 2742.
51. Nagafuchi Y, Shoda H, Fujio K. Immune profiling and precision medicine in systemic lupus erythematosus. *Cells* 2019; 8 (2): 140.
52. Benedek G, Meza-Romero R, Bourdette D, Vandenbark AA. The use of flow cytometry to assess a novel drug efficacy in multiple sclerosis. *Metab Brain Dis* 2015; 30 (4): 877-84.
53. Ashihara E, Shimazaki C, Okano A *et al*. Infusion of a high number of CD34+ cells provides a rapid hematopoietic recovery and cost savings in autologous peripheral blood stem cell transplantation. *Jpn J Clin Oncol* 2002; 32 (4): 135-9.
54. Rawat A, Arora K, Shandilya J *et al*. Flow cytometry for diagnosis of primary immune deficiencies: a tertiary center experience from North India. *Front Immunol* 2019; 10: 2111.
55. Kagan JM, Sanchez AM, Landay A, Denny TN. A brief chronicle of CD4 as a biomarker for HIV/AIDS: a tribute to the memory of John L. Fahey. *For Immunopathol Dis Therap* 2015; 6 (1-2): 55-64.
56. Wang X, Li ZY, Zeng L *et al*. Neutrophil CD64 expression as a diagnostic marker for sepsis in adult patients: a meta-analysis. *Crit Care* 2015; 19 (1): 245.
57. Genel F, Atlihan F, Gulez N *et al*. Evaluation of adhesion molecules CD64, CD11b, and CD62L in neutrophils and monocytes of peripheral blood for early diagnosis of neonatal infection. *World J Pediatr* 2012; 8 (1): 72-5.
58. Robinson JP, Ostafe R, Iyengar SN *et al*. Flow cytometry: the next revolution. *Cells* 2023; 12 (14): 1875.
59. Nagwa AE, Ahmed MM. Significance of CD133 expression in invasive ductal carcinoma of the breast. *Asian Pac J Cancer Biol* 2022; 7 (1): 9-14.
60. Wang X, Li ZY, Zeng L *et al*. Neutrophil CD64 expression as a diagnostic marker for sepsis in adult patients: a meta-analysis. *Crit Care* 2015; 19 (1): 245.
61. Genel F, Atlihan F, Gulez N *et al*. Evaluation of adhesion molecules CD64, CD11b, and CD62L in neutrophils and monocytes of peripheral blood for early diagnosis of neonatal infection. *World J Pediatr* 2012; 8 (1): 72-5.
62. Robinson JP, Ostafe R, Iyengar SN *et al*. Flow cytometry: the next revolution. *Cells* 2023; 12 (14): 1875.
63. Abd El-Sadek N, Mohammed AMH. Significance of CD133 expression in invasive ductal carcinoma of the breast. *Asian Pac J Cancer Biol* 2022; 7 (1): 9-14.