A COMPREHENSIVE REVIEW ON CANCER IMMUNOTHERAPY IN VETERINARY MEDICINE

ABSTRACT

According to a World Health Organization (WHO) estimate, cancer is one of the leading causes of mortality worldwide, expected to claim the lives of almost 10 million people in 2020. It is estimated that 30% of cancer incidences in low- and lower-middle-income countries are caused by diseases like hepatitis and HPV. Many tumours are treatable if detected early and treated properly. The most common malignancies in dogs include transmissible venereal tumours, mammary gland tumours (breast cancer), spleen cancer, skin cancer, lymphatic cancer, gum tumours (epulis cancer), and eye cancer. The frequency of canine cancer cases in India has been rising quickly.

The fast-developing fields of cancer immunology and immunotherapy have revolutionized how cancer is seen and treated. The process of immunotherapy involves using the body's immune system to locate and destroy cancer cells. New immunotherapy medications that selectively target cancer cells while sparing healthy cells have been developed as a result of advancements in immunology. The present study reviews the cause, impacts and effects of Cancer along with the feasibility of Immunotherapy as a cure for the cancer.

Checkpoint inhibitors, monoclonal antibodies, vaccinations, and adoptive cell therapy are a few types of immunotherapies. To more effectively target these malignancies, researchers are exploring novel immunotherapy techniques such as oncolytic virotherapy or CAR T-cell treatment. Researchers are working to develop biomarkers to identify which patients would benefit most from immunotherapy.

Different immunotherapies have been used by veterinary medicine researchers to treat canine neoplasms. These include the use of "caninized" mAb against canine PD-L1 in dogs that have metastatic melanoma, IL-12 electrogene treatment for mast cell tumours in dogs, and hTyr-specific T cells for stage II and III melanoma.This review discusses several immunotherapy approaches for cancer and their outcomes.

1. INTRODUCTION

1.1 Key Facts on Cancer

Cancer is a condition in which some cells in the body develop uncontrolled and spread to other regions of the body. These tumours can be malignant or benign (not cancerous).Normal cells may become cancer cells. Before cancer cells form in tissues of the body, the cells go through abnormal changes called hyperplasia and dysplasia. In hyperplasia, there is an increase in the number of cells in an organ or tissue that appear normal under a microscope. In dysplasia, the cells look abnormal under a microscope but are not cancer.Hyperplasia and dysplasia may or may not become cancer.

According WHO report, around 10 million deaths, or roughly one in six deaths, were caused by cancer in 2020, making it the top cause of death globally. In low- and lower-middle-income nations, cancer-causing infections including the human papillomavirus and hepatitis are thought to be the cause of 30% of cancer cases. If caught early and appropriately treated, many tumours are curable.

While 94 cases of cancer in dogs were reported at the Indian Veterinary Research Institute (IVRI), Uttar Pradesh in 2006-07, the number rose to 209 in 2016-17 more than a two-fold growth(1).worked based on a retrospective study of 88 biopsies of dogs received over ten years (2005 to 2014). The result revealed that the highest number of tumours was epithelial followed by mesenchymal and mixed ones. The incidence of dog tumours remained consistent from 2005 to 2013, except for a decline in 2011 due to variable reporting by owners (Fig. 1). The increase in neoplastic disease necessitates continuous development from veterinary oncology specialists.
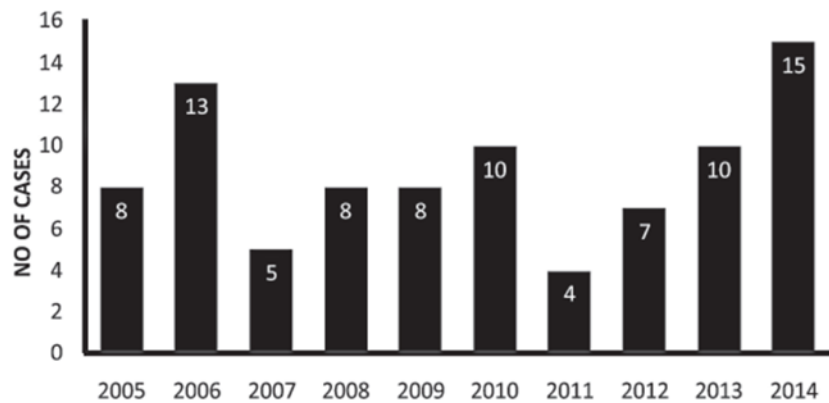
Fig.1. Year-wise incidence of canine tumours from 2005 to 2014 (1)

In the Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry (COVS), Anand, Gujarat, India, a total of 150 biopsy samples and 62 FNAC samples were collected from Canines, Bovines and Equines. Types of cancer in findings were squamous cell carcinoma, TVT, mammary gland tumour, and fibroma. Around 19 cases in dogs were diagnosed with cancer, as detailed in Table 1.

Table 1. Annual progress report by COVS, Anand, 2022-23

Sr. No.	Breed	Total	
		Number	Percentage (%)
1	Labrador Retriever	7	36.84
2	Doberman Pinscher	3	15.78
3	German Shepherd	3	15.78
4	Pomeranian	3	15.78
5	Rottweiler	2	10.52
6	Mongrel	1	5.26
	Total	**19**	**100**

According to the American Veterinary Medical Association (AVMA), Neoplasia affects about 1 in 4 dogs at some point in their lives. The incidence of cancer in dogs over the age of 10 is about 50%. According to preliminary estimates of cancer incidence by the National Cancer Institute's Center for Cancer Research (CCR), there are roughly 6 million new cancer diagnoses made each year in dogs and a comparable amount in cats. The incidence of cancer in dogs is nearly the same as that in humans, however, less is known about the incidence of cancer in cats. Some cancers affect cats more frequently than dogs, like lymphoma.

1.2 The Role of Comparative Oncology

Recently, research on cancer in pets is changing our understanding of human cancers and leading to new treatment choices. According to an article by (2), the spontaneous malignancies in pets treated by veterinary oncologists are identical to those in humans. The only two species that naturally produce deadly prostate tumours are dogs and humans. Breast cancer in dogs, like breast cancer in humans, spreads to the bones. and Osteosarcoma, the most common bone cancer in dogs, is the same illness that affects teenagers.

2. CELL CYCLE AND IT'S CHECKPOINTS

A checkpoint is one of several locations in the eukaryotic cell cycle (Fig. 2) where a cell might stop progressing to the following stage of the cycle until circumstances are favourable. These checkpoints take place during metaphase, at the G2/M transition, and after G1. The daughter cells that are created must be identical replicas of the parent cell. Errors in chromosomal duplication or distribution result in mutations that might be carried over to every new cell generated from an aberrant cell. There

52 are internal regulatory mechanisms that function at the three main cell cycle checkpoints to stop a
53 damaged cell from continuing to divide. There are checkpoints built into the cycle to ensure everything
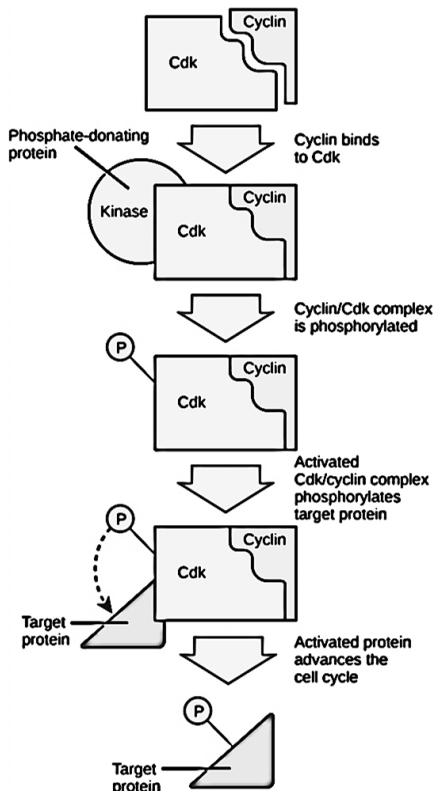54 goes according to plan.
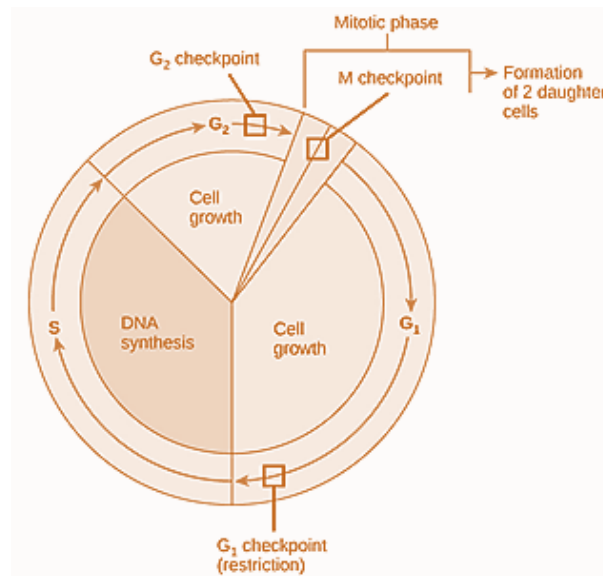

Fig. 2. Cell cycle(3)

55 In addition to the internally controlled checkpoints, the cell cycle is regulated by two groups of
56 intracellular molecules. These regulatory chemicals can stimulate cell progression to the next phase
57 (positive regulation) or inhibit cell progression (negative regulation). Regulator molecules can either
58 operate independently or impact the activity or synthesis of other regulatory proteins. As a result, the
59 failure of a single regulator may have little impact on the cell cycle, especially if many mechanisms
60 control the same event. The consequence of a poor or non-functioning regulator, on the other hand,
61 can be wide-ranging and even lethal to the cell if many processes are disrupted.

62 The progression of the cell through multiple checkpoints is regulated by two protein families known as
63 cyclins and cyclin-dependent kinases (Cdks). Throughout the cell cycle, the four cyclin proteins' levels
64 change regularly. The cyclins that were active in the preceding stage of the cell cycle are destroyed
65 after the cell advances to the following stage.

66 The Cdk/cyclin complex must also be phosphorylated in particular sites to be completely active (Fig.
67 3). Cdks, like all kinases, are enzymes that phosphorylate other proteins. Phosphorylation activates
68 proteins by altering their structure. Cdk-phosphorylated proteins are important in the cell's progression
69 to the next phase.

Fig. 3. Cyclin-dependent kinases(3)
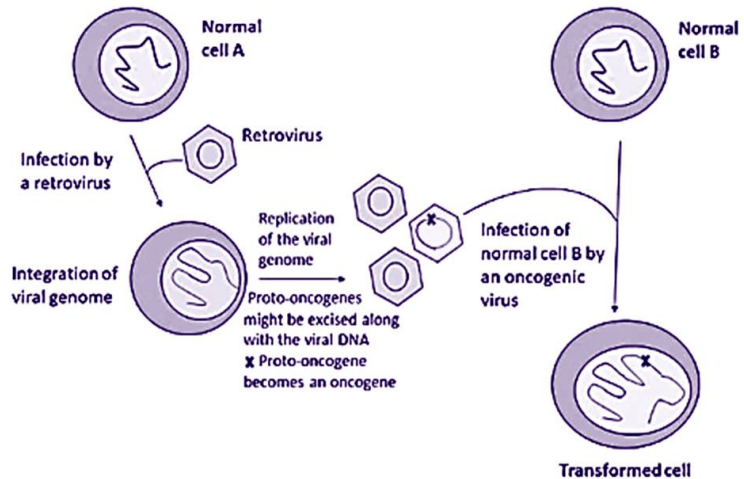

Fig. 4. Proto-oncogenes(4)

70 Negative regulators halt the cell cycle. Retinoblastoma protein (Rb), p53, and p21 are the most well-
71 studied negative regulatory molecules. Retinoblastoma proteins are a type of tumour suppressor
72 protein found in a variety of cells. The numbers 53 and 21 relate to the functional molecular masses
73 (p) of the proteins in kilo Daltons. All three of these regulatory proteins were found to be damaged or
74 non-functional in malignant cells that had begun to multiply uncontrolled. The main reason for the
75 unregulated progression through the cell cycle in each case was a defective copy of the regulatory
76 protein.

77 Through the transactivation of its target genes involved in the induction of cell cycle arrest and/or
78 apoptosis, activated p53 promotes cell cycle arrest to allow DNA repair and/or death to limit the
79 proliferation of cells with substantial DNA damage.The genes that code for the positive cell cycle
80 regulators are called proto-oncogenes. Proto-oncogenes are normal genes that, when mutated in
81 certain ways, become oncogenes, genes that cause a cell to become cancerous (Fig. 4).

82 3. CANCER AND IMMUNE SYSTEM

83 A network of biological mechanisms called the immune system guards an organism against illness. It
84 recognises and reacts to a wide range of pathogens, separating them from the organism's healthy
85 tissue, including viruses, parasitic worms, cancer cells, and things like wood splinters. Many animals
86 have two key subsystems of the immune system. The innate immune system responds to a variety of
87 events and stimuli in a predetermined way. The adaptive immune system learns to recognise
88 chemicals it has previously met and responds to each stimulus in a way that is specific to that
89 stimulus. Both rely on molecules and cells to carry out their tasks.

90 Malignant tumour growth and progression have been demonstrated to be controlled by adaptive
91 immune responses, which are mostly mediated by T cells. Patients and experimental animals can
92 show both innate and adaptive immune responses, and different immune pathways can kill tumour
93 cells in vitro. The difficult task for tumour immunologists is to identify which of these mechanisms may
94 significantly contribute to the defence against tumours and to improve these effector mechanisms in
95 tumour-specific ways.
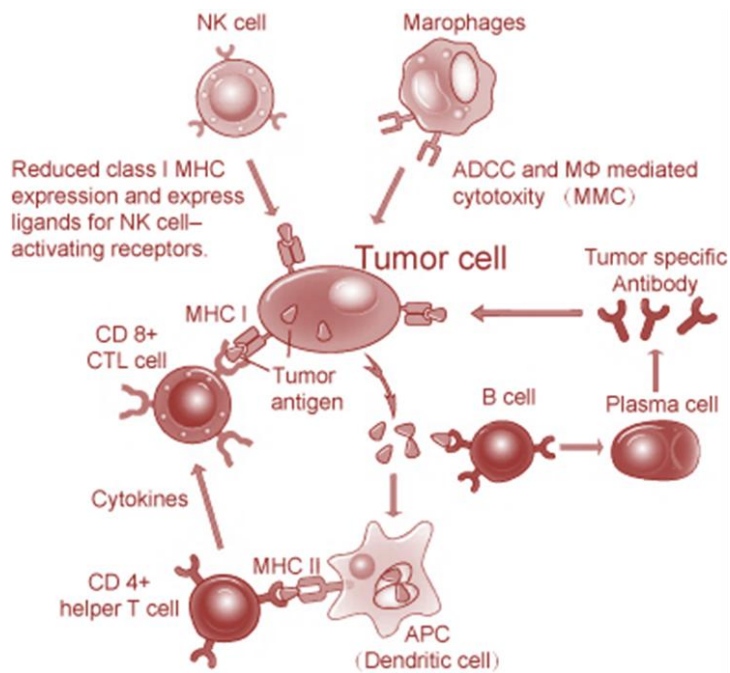
Fig. 5. Immune response against tumour(5)

3.1 CD8+ T and CD4+ T Lymphocytes

"The destruction of tumour cells by CD8+ CTLs is the primary mechanism of adaptive immune defence against tumours. CTLs may operate as a surveillance system, recognising and destroying possibly malignant cells that produce peptides derived from tumour antigens and displayed in conjunction with class I MHC molecules"(6)

"CD4+ cells may contribute to anti-tumor immune responses by supplying cytokines for the development of naïve CD8+ T cells into effector and memory CTLs. Furthermore, helper T cells specific for tumour antigens may produce cytokines such as TNF and IFN-, which might boost tumour cell class I MHC expression and susceptibility to CTL lysis"(6)
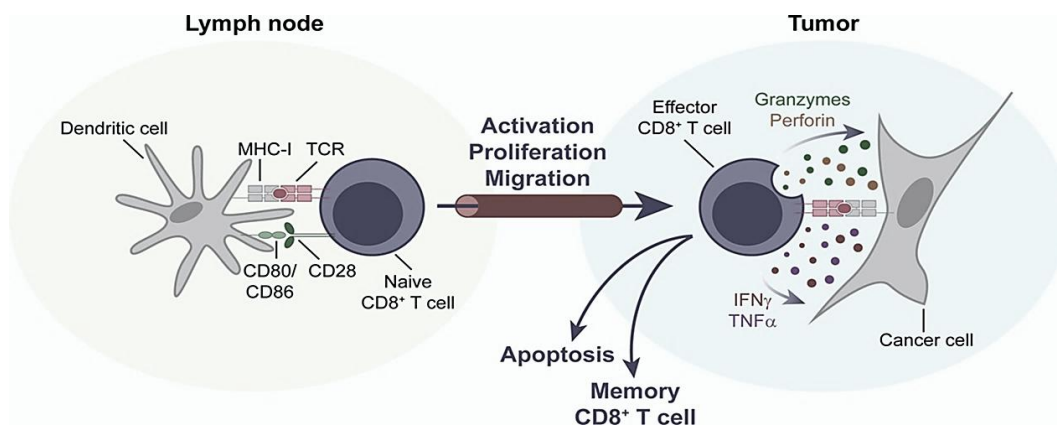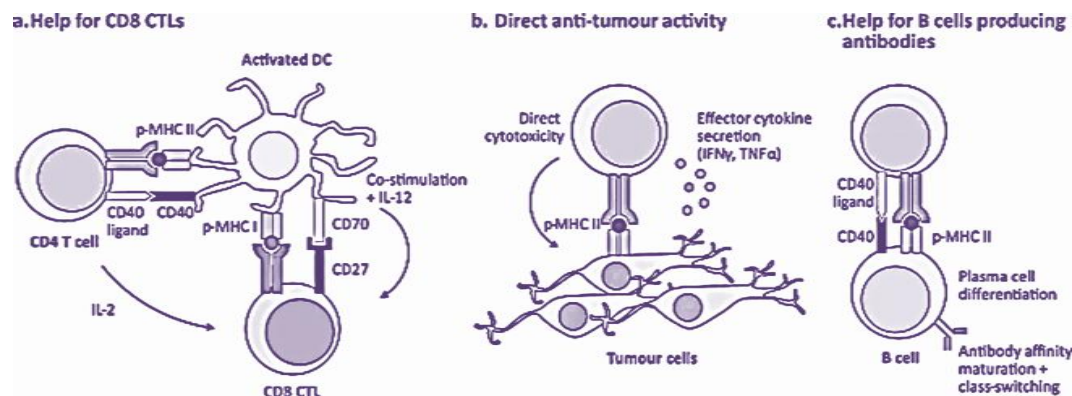

Fig. 6. CD8+ T Lymphocytes (6)

Fig. 7. CD4+ T Lymphocytes (6)

3.2 Antibodies, Natural Killer Cells and Macrophages

"Tumour-bearing hosts may produce antibodies against various tumour antigens. Antibodies may kill tumor cells by activating complement or by antibody-dependent cell-mediated cytotoxicity, in which FcR–bearing macrophages or NK cells mediate the killing. However, the ability of antibodies to eliminate tumour cells has been demonstrated largely in vitro, and there is little evidence for effective humoral immune responses against tumours"(Cooper et al., 2001)

Natural Killer (NK) cells destroy a wide variety of tumour cells, particularly those that produce ligands for NK cell activation receptors and have decreased class I MHC expression. Uncertainty surrounds the role of NK cells in tumour immunity in vivo. According to several research, mice without T cells do not develop spontaneous tumours as frequently, and this is ascribed to the existence of normal levels of NK cells, which have an immunological surveillance role.

Tumor cells can be inhibited or promoted by macrophages, depending on their activation state. M1 macrophages can kill tumour cells, while M2 macrophages contribute to tumour progression by secreting VEGF, TGF-β, and other factors that promote tumour angiogenesis. The activation state of macrophages by tumours remains unknown.
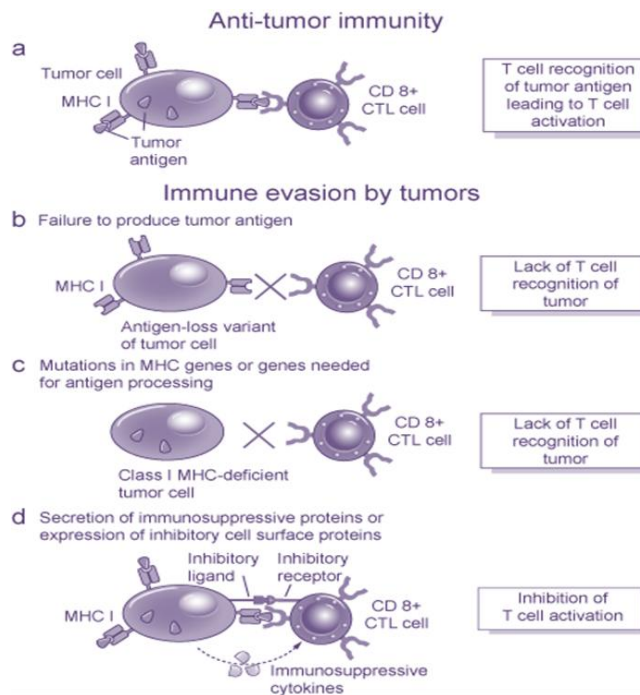
3.3 Evasion of immune responses by tumours

1. "Escaping Immune Recognition by Loss of Antigen Expression
Tumour cells undergo tumour immunoediting, a process where immune responses pressure them to survive and outgrow variant cells with reduced immunogenicity. This is due to mutations or deletions in genes encoding tumour antigens, which can give antigen-negative tumour cells an advantage over the host immune system"(8). Tumour cells also show decreased synthesis of class I MHC molecules, β2-microglobulin, and antigen processing machinery, which may allow them to evade T cell-mediated immune responses (Fig. 8).

2. Active Inhibition of Immune Responses
T cells play a crucial role in suppressing immune responses to tumours, with CTLA-4 and PD-1 being key inhibitory pathways. Tumour-associated macrophages may also promote tumour growth and invasiveness by altering the tissue microenvironment and suppressing T-cell responses. Myeloid-derived suppressor cells also play a role.

Anti-tumor immunity

a

Tumor cell

MHC I

Tumor antigen

CD 8+ CTL cell

T cell recognition of tumor antigen leading to T cell activation

Immune evasion by tumors

b Failure to produce tumor antigen

MHC I

CD 8+ CTL cell

Antigen-loss variant of tumor cell

Lack of T cell recognition of tumor

c Mutations in MHC genes or genes needed for antigen processing

CD 8+ CTL cell

Class I MHC-deficient tumor cell

Lack of T cell recognition of tumor

d Secretion of immunosuppressive proteins or expression of inhibitory cell surface proteins

Inhibitory ligand   Inhibitory receptor

MHC I

CD 8+ CTL cell

Immunosuppressive cytokines

Inhibition of T cell activation

Fig. 8. Evasion of Immune Responses by Tumors(5)

134 **3.4Types of Cancer Treatment**

135 Many procedures and drugs are available to treat cancer, with many more being studied. Some are
136 "local" treatments like surgery and radiation therapy, which are used to treat a specific tumour or area
137 of the body. Drug treatments (such as chemotherapy, immunotherapy, or targeted therapy) are often
138 called "systemic" treatments because they can affect the entire body.

139 **4. IMMUNOTHERAPY**

140 Immunotherapy is a form of cancer treatment that makes use of the patient's immune system.
141 Immunotherapy can alter or enhance the immune system's functioning to enable it to locate and
142 destroy cancer cells

143 **4.1Immune Checkpoint Inhibitors**

144 "The immune system's capacity to distinguish between bodily normal cells and those it perceives as
145 "foreign" (such as pathogens and cancer cells) is a crucial component of the immune system. As a
146 result, the immune system can target foreign cells while sparing healthy ones. The immune system
147 uses "checkpoint" proteins on immune cells as one method of accomplishing this. These checkpoint
148 proteins can be the target of monoclonal antibodies, which are types of medications"(9).

149 Immune checkpoint inhibitors (or simply checkpoint inhibitors) are the name given to these
150 medications. Checkpoint inhibitors do not directly kill cancer cells. They function by facilitating the
151 immune system's detection and destruction of cancer cells throughout the body. To initiate an immune
152 response, the checkpoints function as switches that must be switched on (or off). However, cancer
153 cells occasionally manage to circumvent these checkpoints to protect themselves from immune
154 system attacks. All of these medications are infused intravenously (IV).

155 James P. Allisonand.TasukuHonjowon jointly won the Nobel Prize in Physiology or Medicine in
156 2018for their discovery of cancer therapy by inhibition of negative immune regulation(10). James P.
157 Allison investigated a well-known protein that restrains the immune system. He saw that if we could
158 let go of the brake, our immune system would be free to go after malignancies. He then expanded on
159 this idea to create a whole new method of patient care.

160 Simultaneously, TasukuHonjo identified a protein on immune cells and, upon a meticulous
161 investigation of its function, ultimately disclosed that it functions as a brake as well, although in
162 another manner. His findings led to the development of remarkably successful cancer
163 treatments.Allison and Honjo demonstrated the many approaches that may be employed in cancer
164 treatment to suppress the immune system's brakes.

165 **4.2PD-1, PD-L1, CTLA-4 and LAG-3 inhibitors**

166 "A checkpoint protein on immune cells known as T cells is termed PD-1. It typically functions as a kind
167 of "off switch" to prevent the T cells from attacking other bodily cells. When it binds to the protein PD-
168 L1 on some normal (and malignant) cells, it accomplishes this. In essence, the T cell is instructed to
169 leave the other cell alone when PD-1 attaches to PD-L1"(11).

170 Large concentrations of PD-L1 in some cancer cells enable them to evade an immunological
171 response. Blocking this binding and enhancing the immune response against cancer cells is possible
172 with monoclonal antibodies that either target PD-1 or PD-L1. (Coy et al.,2017); Poole (2014);(14);(15–
173 18)

174 Examples of drugs that target PD-1 include:
175   • Pembrolizumab (Keytruda)
176   • Nivolumab (Opdivo)
177   • Cemiplimab (Libtayo)
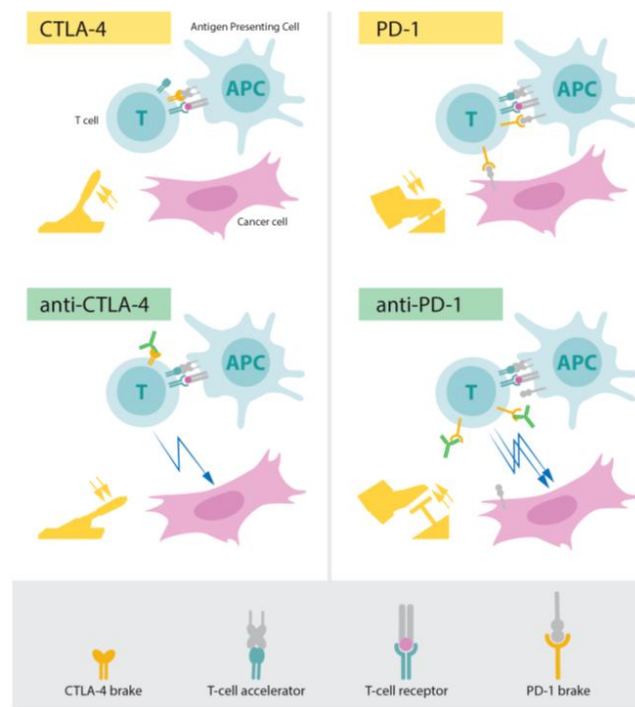
Fig. 9.Upper left: Activation of T cells requires that the T-cell receptor binds to structures on other immune cells recognised as" non-self". A protein functioning as a T-cell accelerator is also required for cell activation. CTLA-4 functions as a brake on T cells that inhibits the function of the accelerator.

Lower left: Antibodies (green) against CTLA-4 block the function of the brake leading to activation of T cells and attack on cancer cells.

Upper right: PD-1 is another T-cell brake that inhibits T-cell activation

Lower right: Antibodies against PD-1 inhibit the function of the brake leading to activation of T cells and highly efficient attack on cancer cells (19).

178

179 "Another checkpoint protein known as CTLA-4 is found on some T cells and functions as a sort of "off
180 switch" to restrain the immune system. Monoclonal antibodies, such as ipilimumab (Yervoy) and
181 tremelimumab (Imjuno), bind to CTLA-4 and prevent it from functioning. This may strengthen the
182 immune system's defences against cancerous cells. PD-1 or PD-L1 inhibitors are frequently used in
183 combination with these medications. Several different cancers can be treated with these
184 mixtures".(18,20–24)

185 On some types of immune cells, LAG-3 (lymphocyte activating gene-3) is a checkpoint protein that
186 typically functions as an "off switch" to restrain the immune system. A monoclonal antibody called
187 relatlimab binds to LAG-3 and inhibits its function. This may strengthen the immune system's
188 defences against cancerous cells.

189 This medication is administered in conjunction with the PD-1 inhibitor nivolumab (Opdualag). In
190 addition to being researched for use in treating various other cancer types, it can be used to treat skin
191 cancer called melanoma.

192 **4.3 Monoclonal Antibodies**

193 Monoclonal antibodies are man-made proteins that function in the immune system similarly to human
194 antibodies.(25–29) To treat CD20-positive B cell (Blontress®) or CD52-positive T cell lymphoma, the
195 US Department of Agriculture has approved the use of monoclonal antibodies.In the USA and
196 Canada and are marketed under the brand name (Tactress®) (30)

197 They can be manufactured in four distinct ways and are named based on what they are composed of:

198 - Murine: These therapies are created from mouse proteins, and their names end in –omab.
199   Chimeric proteins are a cross between mouse and human proteins, and the names of the
200   medicines finish in –ximab.
201 - Humanized: These medicines are constructed from small bits of mouse proteins linked to
202   human proteins, and their names end in –zumab.
203 - Human: These are human proteins, and the medicines' names finish in –umab.
204
205 **4.3.1 Types of Monoclonal Antibodies**

206   1. **Conjugated monoclonal antibodies:**

207   Conjugated mAbs are made up of either a radioactive particle or a chemotherapeutic
208   medication. Tagged, labelled, or loaded antibodies are other names for conjugated
209   monoclonal antibodies (mAbs).

210   The antibody directly delivers radioactivity to cancer cells. It is composed of a mAb
211   medication (rituximab) as well as a radioactive material (Yttrium-90). Radiolabeled mAbs
212   include ibritumomabtiuxetan (Zevalin). This is an antibody against the CD20 antigen, which is
213   found on B cells, which are lymphocytes.

214   2. **Bispecific monoclonal antibodies:**

215   These medications are composed of portions of two separate mAbs, allowing them to bind to
216   two different proteins at the same time. Blinatumomab (Blincyto), for example, is used to treat
217   some kinds of leukaemia.

218   One component of blinatumomab binds to the CD19 protein present in some leukaemia and
219   lymphoma cells. Another component binds to CD3, a protein found in immune cells known as
220   T cells. This medicine, by attaching to both of these proteins, brings cancer cells and immune
221   cells together, causing the immune system to kill the cancer cells.

222 **4.4 CAR T-cell Therapy**

223 "Chimeric antigen receptor (CAR) T-cell therapy is a method of directing immune cells known as T
224 cells (a kind of white blood cell) to fight cancer by modifying them in the lab so that they can detect
225 and destroy cancer cells"(31). Because it includes changing the genes inside T cells to help them
226 attack cancer, CAR T-cell therapy is sometimes referred to as a sort of cell-based gene therapy. ((32–
227 39)

228 This form of treatment can be quite beneficial in the treatment of certain types of cancer, even when
229 other treatments are no longer effective. Because various tumours have distinct antigens, each CAR
230 is tailored to that cancer's antigen.

231 "For example, cancer cells in certain types of leukaemia or lymphoma have an antigen called CD19.
232 CAR T-cell treatments for these tumours are designed to connect to the CD19 antigen and will not
233 work on tumours that lack the CD19 antigen". ((39–41)
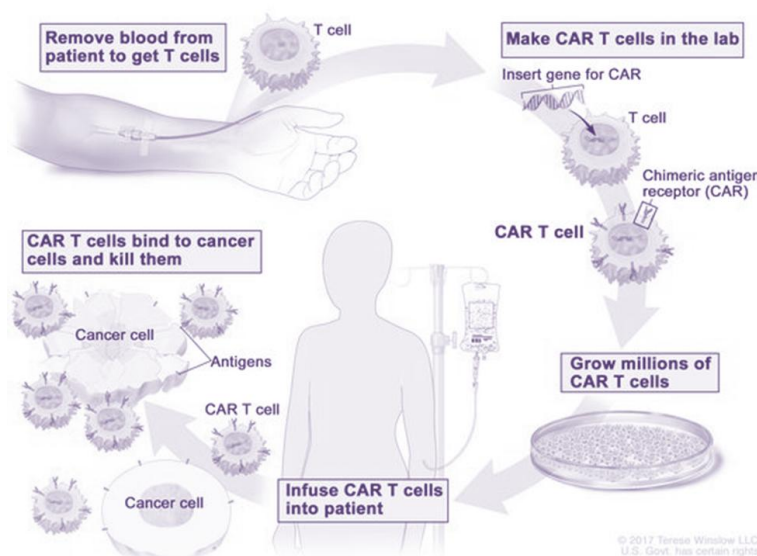
Fig.10. CAR T-cell Therapy(42)

234   **4.5 Cytokines**

235 "Cytokines are small proteins that regulate the growth and function of other immune systems and
236 blood cells. They alert the immune system to start working when they are released. They also
237 contribute to anti-cancer activities by transmitting signals that cause abnormal cells to perish and
238 normal ones to live longer"(43)

239 A chemokine is a specific sort of cytokine. A chemokine can direct immune cells toward a certain
240 target. Chemokines are classified into several types, including interleukins, interferons, tumour
241 necrosis factors, and growth factors. Cytokines influence the formation of all blood cells and other
242 cells that aid in the immunological and inflammatory responses of the body.

243 Interleukins: Interleukins are cytokines that serve as chemical messengers between white blood cells.
244 Interleukin-2 (IL-2) promotes the growth and division of immune system cells. A synthetic form of IL-2
245 has been approved for the treatment of advanced kidney cancer and metastatic melanoma. For these
246 tumours, IL-2 can be given alone or in combination with chemotherapy or other cytokines such as
247 interferon-alfa.

248 Interferons: Interferons are substances that aid the body's defences against virus infections and
249 cancer. Interferon (IFN) types are called after the first three letters of the Greek alphabet: IFN-alfa,
250 IFN-gamma & IFN-beta. Cancer is only treated with IFN-alfa.

251   **4.6 Cancer vaccines**

252 Vaccines are substances put into the body to start an immune response against certain diseases.
253 Usually, vaccines are thought to be given to healthy people to help prevent infections. But few
254 vaccines can help prevent or treat cancer.

255 4.6.1 Dendritic cell vaccines

256 A novel treatment approach for cancer patients is the dendritic cell-based cancer vaccination. To
257 trigger an antigen-specific T-cell response, dendritic cells (DCs) transmit antigens to the body.

258 Immunotherapy based on DCs is a safe and effective way to support immune responses against
259 tumours and extend cancer patients' lives. Inducing cellular immunity is the primary objective of
260 therapeutic vaccinations. Priming naïve T cells and causing the shift from chronically activated non-
261 protective CD8+ T cells to healthy CD8+ T cells is important because they can generate cytotoxic T
262 lymphocytes (CTLs), which can identify and destroy cancer cells by identifying certain antigens. Long-
263 lived memory CD8+ T cells are also produced by this process, and they will function to stop relapses.

264 The most important stage in vaccination is successfully presenting cancer antigens to T cells. Since
265 DCs are highly potent cells to deliver antigens, they provide a viable alternative for enhancing
266 therapeutic vaccinations(44).
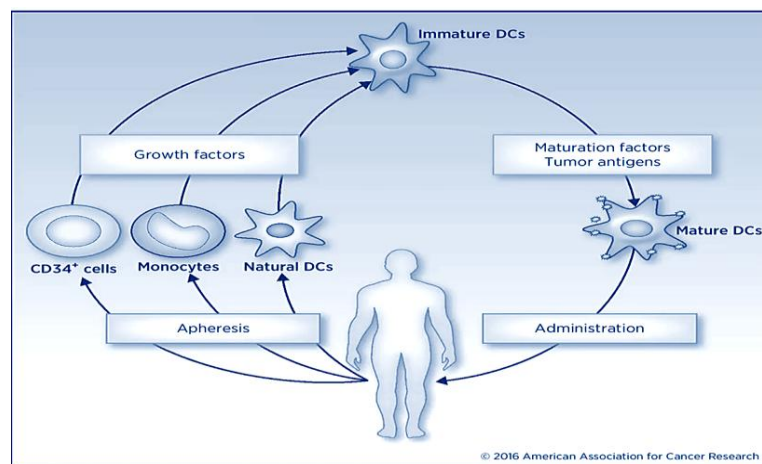
Fig. 11.Dendritic cell vaccines(45)

267 4.6.2 Oncolytic Virus

268 Viral infection and subsequent destruction of cells is known as "oncolytic virus therapy."Viruses are
269 small, invading particles that enter or infect our cells. They then exploit the genetic machinery of the
270 cell to replicate, spreading to neighbouring, uninfected cells.

271 Several viruses, including the human papillomavirus (HPV), which causes the cervical head and neck
272 cancer, and the hepatitis B virus (HBV), which causes liver cancer, have been linked to the
273 development of certain malignancies(46).

274 For several reasons, oncolytic viruses are a potentially effective cancer treatment method:

275   1. Immunodeficiency of cancer cells frequently results in compromised antiviral defences.

276   2. These naturally occurring viruses can be modified to have beneficial traits, such as a reduced
277   capacity to infect healthy cells, the capacity to target tumours with therapeutic payloads, and
278   the capacity to release immune-stimulating compounds once they have infected tumour cells.

279   3. These oncolytic viruses can infect cancer cells and induce them to "burst," releasing cancer
280   antigens and destroying cancer cells. The immune systems that these antigens trigger may
281   then search for and destroy any residual tumour cells in the area and maybe elsewhere in the
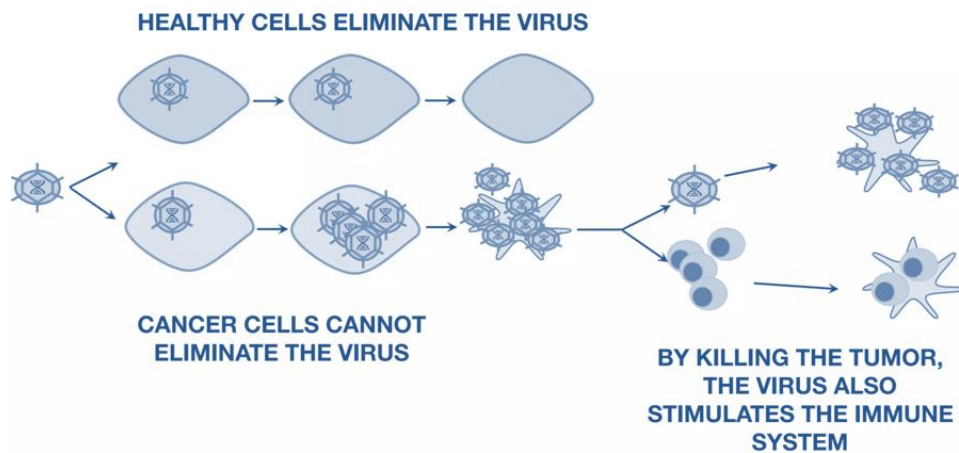282   body.

Fig. 12. Oncolytic viruses(3)

283 **4.7 BTLA/HVEM Axis Dysregulation**

284 "In recent years, interest in B-and T-Lymphocyte Attenuator (BTLA), an inhibitory
285 immunological checkpoint expressed on B, T, and NK cells, has grown. HVEM functions as
286 BTLA's binding partner and is distinguished by a more widespread expression because it is
287 also found in neuronal, hematopoietic, epithelial, and endothelial cells.
288 HVEM functions as a bidirectional switch that may both initiate its signal transduction and
289 act as a ligand for various co-stimulatory and co-inhibitory substances. The preservation of
290 the immune response's equilibrium depends on this implica network"(47)."Chronic
291 lymphocytic leukaemia (CLL) patients develop immunosuppression, affecting their antitumor
292 responses. The BTLA/HVEM axis plays a role in promoting defects in T cell-mediated
293 responses against leukemic cells. Increased BTLA expression on CD4+ and CD8+ T
294 lymphocytes correlates with diminished treatment time. BTLA activation leads to decreased
295 IL-2 and IFN-γ production, while BTLA/HVEM binding disruption enhances IFN-γ+CD8+ T
296 lymphocytes. Treatment with anti-BLTA blocking monoclonal antibodies and ibrutinib can
297 induce leukemic cell depletion. This dysregulation has a prognostic role and limits T-cell-
298 driven antitumor responses"(48).

299 **4.7.1 TIM-3 Immunoregulatory role and its interactions.**

300 T cell immunoglobulin and mucin domain-containing protein 3 (TIM3) is a member
301 of the TIM family that was first shown to be expressed on CD4+ and CD8+ T cells that
302 produced interferon-γ. Because there was no discernible inhibitory signalling motif, it was
303 also hypothesised that TIM3 might operate as a co-stimulatory receptor. Initially, findings
304 suggested that TIM3 operated as a "co-inhibitory" or "checkpoint" receptor. TIM3 is a
305 component of a module that also includes other co-inhibitory receptors, or "exhausted" T
306 cells, which are co-expressed and co-regulated in cancer and persistent viral infections(49)

307

308 **5. CANCER IMMUNOTHERAPY IN VETERINARY MEDICINE**
309 Veterinarian immunotherapy is not as advanced as it might be, and there are several reasons why,
310 the most important being financial. Biotechnology companies are aware that the veterinary industry
311 has limited access to costly immunotherapies; as a result, they are hesitant to contribute enough
312 funding for clinical studies, which can be prohibitively expensive due to the immunotherapy's high
313 costs. Immunotherapy is a significant challenge to the area of veterinary medicine due to the lack of
314 comprehensive evaluation of the canine immune system and the unknown cellular biology of tumours
315 in dogs.Even if the previous several years have seen an increase in clinical studies, financial support,
316 and attitudinal changes, we are still a long way off.

317

Table 2. Advancements in Cancer Immunotherapy

Advancements	Reference
hTyr-specific T cell (plasmid DNA encoding human tyrosinase) permitted for the treatment of stage II and III melanoma	(50)
In canine transmissible venereal tumour (TVT), IL-12 electrogene treatment resulted in a considerable delay in tumour development and subsequently, total tumour remission	(51)
Interlekin-12 (IL-12) stimulates NK cells and causes IFN-gamma to be released. Numerous clinical studies testing the efficacy of IL-12 gene therapy in various veterinary animals have been conducted	(52,53)
In dogs with mast cell tumours, IL-12 electrogene treatment led to a 50% decrease in tumour volume	(54)
PDL1 expression has been found on a variety of canine tumours in studies employing canine tumour biopsy samples and a human mAb with cross-reactivity with canine PD-L1	(17)
In dogs with metastatic melanoma, a recent clinical trial employing a "canonised" mAb against canine PD-L1 showed a limited response	(14)
The United States Department of Agriculture recently granted full (CD20) or conditional (CD52) licenses for clinical use in the treatment of canine B-cell and T-cell lymphomas, respectively	(30)

318

6. CONCLUSIONS

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320 Various studies indicate that year-wise incidence of cancer is increasing in dogs and cats. In India,
321 the number of canine cancer cases has been rapidly increasing due to a lack of systematic research
322 and an animal cancer registry.

323 The cell cycle checkpoints take place during metaphase, at the G2/M transition, and after G1, to
324 ensure whether the cell cycle goes normal or not.T cells play a central role in immune responses to
325 cancer.Immunotherapy is a form of cancer treatment that uses the patient's immune system to identify
326 and combat cancer cells.Types of Immunotherapies are checkpoint inhibitors, monoclonal antibodies,
327 CAR T-cell therapy, Cancer vaccines and Oncolytic viruses.

328 In Veterinary medicine, the use of Immunotherapeutic agents is increasing day by day i.e. IL-12 gene
329 therapy, "caninized" mAb against canine PD-L1, and hTyr-specific T cell therapy in canine
330 cancer.Tumour immunotherapy is most likely to be acknowledged as the fourth weapon in the battle
331 against cancer in veterinary medicine soon, joining surgery, chemotherapy, and radiation treatment,
332 given the growing interest and modern research in the field.

7. FUTURE PROSPECTS

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334 There are still many cancer types that do not react to existing immunotherapy treatments, but
335 researchers are striving to understand how cancer cells interact with the immune system and create
336 novel treatments to stop cancer from spreading.We can explore ways to combine immunotherapy with
337 other treatments, such as chemotherapy or radiation therapy, to improve outcomes.Nowadays
338 immunotherapy is costly, but it should be cost effective in future.The future scope of cancer in
339 Veterinary medicine is, to establish separate branches of veterinary oncology and immunotherapy for
340 effective treatment.

COMPETING INTERESTS

The authors have declared that no competing interests exist.

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