Cutaneous Melanoma: A Comparative Study Between Gray Horses, Canines, and Humans

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Abstract

Cutaneous melanoma affects multiple species, including gray horses, dogs, and humans. It is typically aggressive in dogs and humans, and more benign in gray horses. Although developmentally different (gray horse melanoma is frequently genetically inherited, while human and canine melanoma is usually acquired), treatment of gray horse, canine, and human cutaneous melanoma is similar. Treatment usually starts with surgical removal, but the best adjuvant treatment is unknown. As additional research is needed to define the best combination treatment approach for all three species, the similarities between them mean they can be translational models for each other. The similarities, differences, and translational opportunities between gray horse, canine, and human melanoma will be explored in the following review.

Introduction:

Skin cancer is common in many species across the globe, manifesting in different forms including melanomas, squamous cell carcinomas, hemangiosarcomas, and mast cell tumors, among others. Cutaneous melanoma causes 55,000 deaths and over 100,000 cases every year, and continues to increase[33]. Human melanoma is defined as a serious form of skin cancer that begins in melanocytes, which come from dendritic cells in the epithelial layer of the skin or mucosa.[29] There are 4 major subtypes that include: Ocular, acral, mucosal, and cutaneous (UV-induced and non-UV-induced types)[2]. This manuscript will focus on the cutaneous, or dermal form. There are 5 stages of human cutaneous melanoma that are categorized below. These 5 stages represent the physical size of the tumor, the depth of tumor penetration, and whether it has metastasized or not[2]:

Stage 0: The cancer is restricted to the epidermis, the first layer of the skin; it has not spread to other tissues or organs, such as the lymph nodes. This stage of melanoma is known as melanoma in situ.

Stage I: The tumor has grown, but is not more than 2 mm thick. At this stage, the tumor may or may not be ulcerated. The cancer continues to remain in the local area and has not spread to local lymph nodes or other tissues of the body.

Stage II: The tumor continues to grow and may be larger than 4 mm thick. It may or may not be ulcerated. The tumor still has not spread to lymph nodes or other tissues in the body.

Stage IIIa: The tumor is no more than 2 mm thick, may or may not be ulcerated, but has spread to 1-3 lymph nodes nearby. It is too small to see grossly (only seen microscopically) and has not spread to distant parts of the body.

Stage IIIb: The primary tumor can no longer be seen and the cancer has spread only to 1 lymph node or it has spread to very limited areas of nearby skin, without reaching the local lymph nodes.

Stage IIIc: The primary tumor continues to show no visible signs grossly and the cancer has spread to 2+ nearby lymph nodes, with at least one visible or felt on palpation. Or the tumor has spread to small areas of the skin nearby, called satellite tumors, or the tumor has invaded lymphatic channels that surround it, then reaching the nearby lymph nodes.

Stage IIId: Tumor is thicker than 4 mm and is ulcerated and has spread to 4+ more nearby lymph nodes or it has spread to nearby lymph nodes that are clumped together. It has also spread to small areas of skin (satellite tumors) or to the lymphatic channels. It has not spread to distant parts of the body.

Stage IV: The tumor can be any thickness and might or might not be ulcerated. The cancer may or may not have spread to local lymph nodes, but it has spread to distant lymph nodes or to organs such as lungs, liver, or brain.

Malignant transformation of melanoma follows a genetic model that results in constitutive activation of oncogenic signal transduction[33]. Upon additional genetic alterations,

benign tumors can mutate into malignancies. Specifically, mutations in $BRAF^{v600}$ and the telomerase reverse-transcriptase (*TERT*) promoter have been linked to the shift from benign to malignant tumors[33]. Malignant melanoma also has the ability to spread to other organs rapidly if not treated at an early stage[14].

It has been discovered that many canine cancers, including melanoma, are similar to their human counterparts in many ways, both histologically and clinically. As such, canine models of melanoma have become a more favorable approach to cancer research, compared to murine models, as they are immuno-competent and develop cancer naturally[32]. Their shorter life-spans also aid in the evaluation of different treatment approaches on disease free intervals and overall survival time in a more expedient manner[32].

Gray horses have also been shown to have a predisposition to melanoma development, although predisposing factors and the pathophysiological development in this species is different from dogs and humans. Specifically, gray horse melanomas usually develop due to a genetic predisposition, not an acquired mutation. In addition, melanomas form close to the epidermis and dermoepidermal junction in dogs and humans, which differs from horses[40]. Looking at these three species instead of just one brings insight to many translational opportunities across multiple medical platforms. Despite significant research into human cutaneous melanomas, our understanding of the disease and the most effective treatment options is still limited. Given the natural occurrence of melanoma in both dogs and gray horses, correlations between the species, and differences, may shed light on the disease in people. Treatments tested in our companion animals today, may lead to cures for people tomorrow. The following manuscript will outline the differences and similarities of cutaneous melanoma between the three species, and offer insight to treatment approaches and prognosis for this cancer.

Gray Horse Melanoma:

Background and Genetic Analysis:

Any breed of horse can produce offspring with gray coloring including Thoroughbreds, the American Quarter Horse, and Arabians, but the gray coat is most pronounced in Lipizzaner and Andalusian breeds. The graying effect can also occur in multiple breeds due to the gray gene being autosomal dominant, requiring only one mutant allele to result in hyperpigmentation of the coat color to gray[39]. When a foal is born heterozygous for this mutation, it will have normal coat color that will eventually become fully gray over time[29]. In Lipizzaner and Andalusian horses, the foals are born with a homozygous gray gene, producing the gray coat when they are born, hence the term a gray horse. In this instance, a gray foal is born with a charcoal, almost black coat[22]. However, the speed of graying, the incidence of vitiligo, and melanoma vary substantially among gray horses[29]. Those that have a homozygous mutation have more rapid graying and become nearly white by the end of the process, compared to horses with heterozygous mutations. Homozygous mutation also leads to a significantly increased risk of cutaneous melanoma compared to heterozygous horses[29].

It has been documented that 80% of gray horses over the age of 15 years old develop cutaneous melanomas and have a decreased survival time when compared to their normally pigmented counterparts[22]. The exact mechanism is unknown, but there are several hypotheses. The gray gene locus contains Syntaxin 17 (*STX17)* gene, but there are an additional three genes that may play a role in the development of gray horse melanoma: *NR4A3* (nuclear receptor subfamily 4, group A, member 3), *TXNDC4* (thioredoxin domain–containing-4′), and *INVS* (inversin)[22]. It has been shown that all genes at this locus (*STX17, NR4A3, TXNDC4,* and *INVS*) are expressed in equine cutaneous melanomas, and *NR4A3* specifically, is overexpressed, while *TXNDC4* and *INVS* are not[29]. This suggests a role for *NR4A3* in the pathogenesis of gray horse melanoma[29]. *NR4A3* is an early response gene that has the ability to act like a transcriptional activator. *NR4A4* controls the regulation of multiple cellular processes such as: proliferation, survival, metabolism, inflammation, differentiation of varied types of cells[7], cell cycle regulation, apoptosis, and carcinogenesis[29]. It is thought that the upregulation of *STX17* and *NR4A3* contribute to the gray phenotype in horses by promoting melanocyte proliferation. The impact of *STX17*, specifically, on melanoma development is less clear, but may play a role in RAS cell signaling, a pathway that has been found to be mutated in more than 50% of human melanomas[29].

Apart from *STX17,* gray horses have also been found to carry a recessive black allele caused by a deletion in exon 2 of the gene *ASIP* (agouti signaling protein)[Rieder]. It is thought that *ASIP*, along with *MC1R* (encodes for melanocortin-1 receptor), are the two most influential genes controlling coat color in the horse[35]. Through human intervention and domestication of horses, multiple coat colors have developed[35]. Seven phenotypes in particular have been studied: gray, bay, dark bay, black, brown, white, and chestnut[35]. Interestingly, it was found that horses with a mutated *ASIP,* particularly homozygotes, were more likely to develop cutaneous melanomas[22]. Agouti signaling through ASIP reduces MC1R activation. MC1R signaling activation promotes melanocyte proliferation, and thus in those horses with ASIP mutations and more MC1R signaling, one might expect increased melanoma development[22]. A study looked at MC1R and ASIP in white horses and discovered that the homozygous dominant genotype for MC1R was not in white horses, and the homozygous recessive genotype was not in black horses[35]. These results confirmed that MC1R does have an affect on coat color through melanogenesis and that the E^E (dominant) allele enhances the process while E^e (recessive) allele inhibits it[35]. This effect, however, was not found to impact rate of graying, speckling, or vitiligo, nor was it as strong an effect when compared to the gray locus (*STX17, NR4A3, TXNDC4,* and *INVS)* impact on melanoma development[Peilberg].

Clinical Presentation and Diagnosis:

As previously mentioned, melanomas in gray horses are common[22]. Development begins around the same time dark coats are transformed into a light gray color, often with vitiligo-like patches[32]. The most common places melanomas form are around the base of the tail, around the

anal/perianal/genital regions, as well as on the face, lips, eyelids[32], base of ears, and along the length of the neck and jugular groove[4].

The masses appear as small palpable nodules, but can often grow in more invasive tumors, which can impact normal functions. For example, large masses on the neck may compress the trachea and/or the esophagus, causing difficulty breathing or swallowing.[4]. Horses tend to develop dermal melanomas from age 1 to 6, and often are not metastatic at the time of diagnosis[21]. Diagnosis of a cutaneous melanoma begins with a biopsy and histopathology. If the tumor is small, a full excisional biopsy can be done. If it is large, a punch biopsy is done. Occasionally, immunohistochemical analysis is required to diagnose cutaneous melanomas[20]. Immunohistochemical analysis looks for specific antigens present on tumor cells, and helps to identify tumor-specific antigens, enzymes, oncogenes, tumor suppressor genes, and tumor cell proliferation markers[8]. This information also helps to diagnose a specific tumor, and often provides prognostic information[8]. Characterizing these biomarkers, one is able to tell if the cancer is benign or malignant, what stage the cancer has progressed to, and allows identification of the cell type and the origin of a mass[8].

Evaluation of internal organs is essential to determine if the melanoma has moved to other areas of the body. If the melanomas were to metastasize and/or develop in or around internal structures, the horse has a higher chance of losing the function of the specific vital organ(s). The most common sites for metastatic disease include the lymph nodes, spleen, liver, skeletal muscle, lungs, and surrounding blood vessels[21]. Indications that the disease has metastasized include facial distortions (due to disease in the nasal cavity), and an increase in colic episodes, with a short time frame between each episode [4]. Frequent colic leads to weight loss and chronic gastrointestinal compromise because the metastatic melanomas take away the vital organs ability to release built up gas and digest food[4]. Once a diagnosis has been confirmed, and the patient evaluated for metastatic disease, treatment options can be discussed.

Treatment:

The mainstay of treatment for gray horse melanoma is surgical removal[22]. Attaining clean surgical margins typically results in long term local control, with a low recurrence rate[22]. For tumors that are incompletely excised with surgery alone, or for those where surgery is not an option, alternative treatment options must be considered, including cryotherapy, intratumoral chemotherapy, electrochemotherapy, hyperthermia, radiation therapy, immunotherapy, biologic response modifiers (cimetidine), and vaccinations.

Cryotherapy can be used alone or as an adjuvant to traditional surgical removal. The process uses extreme cold temperatures generated by pressurized liquid nitrogen to destroy tissue[10][30]. The procedure can be broken down into two mechanisms of how the tissue is damaged. The first is initiating ischemia into the tissues by damaging blood capillaries and vessels in a specific area which ultimately causes ischemic necrosis in the tissue[31]. The second mechanism injures the cells more discreetly by forming ice crystals and causing osmotic cell injury and disrupting the cell membrane[31]. As the tissues cool, the ice crystals achieve two

outcomes. They first create an osmotic gradient that will quickly push water out of the cell and then the crystals will form inside of the cell, eventually causing the cell to rupture and die[31]. Another way the crystals damage the cell is during the thawing process. If the cell has not died from cell membrane malfunctions, when thawed, the crystals that formed outside of the cell, melt, and generate a gradient that will draw water back into the cell which will lead to cell swelling and eventually cell death[31]. The advantage of doing cryotherapy versus excision of the lesion is that with excision, the host no longer is exposed to the antigens that are presenting themselves on the malignant cells, but with cryotherapy the antigens on the dead malignant cell stay within the host allowing its immune system develop a response which could lead to a systemic response towards other malignant cells[31]. Cryotherapy should not be done on neoplasms with undetermined behavior because cryotherapy can induce vasoconstriction, which could lead to serious conditions[31]. Cryotherapy is not a cure, but has been shown to manage benign and some malignant tumors. Possible side effects include tissue damage, pain, scarring, alopecia, additional depigmentation, and pseudoepitheliomatous hyperplasia[31]. Overall, cryotherapy is efficient, cost-effective, and has a decent ability to treat both benign and malignant tumors. It takes several sessions and is a way to maintain health and quality of life, but it will not cure gray horse melanoma.

Hyperthermia is a treatment that may accelerate the rate of anti-tumor effects after cryosurgery. Hyperthermia has several mechanisms of action. When the surrounding tissues are heated to 105°F - 110℉, tumor cells succumb to heat related stress[22]. Heat-stressed tumor cells overproduce heat-shock proteins and shed molecules of damage-associated molecular patterns (DAMPs)[22]. The host's antitumor immune responses are elevated[22]. Heat can also intensify the cytotoxicity of chemotherapeutic agents by reversibly permeabilizing tumor infected cell membranes and inhibiting the cells DNA repair mechanisms[22]. Thus, this procedure is often combined with local intratumoral injections of cisplatin[28]. Interestingly, the heat does not affect normal cells as they are able to adapt to the temperature.

Intratumoral chemotherapy with cisplatin or carboplatin is an alternative treatment option. Cisplatin and carboplatin are platinum-based chemotherapy drugs used to treat a variety of cancers[4]. *In vivo,* the platinum complexes bind and cause crosslinks with DNA[38i]. This formation leads to DNA replication failure and eventually cell death[38]. Cisplatin and carboplatin are aqueous solutions, and thus are able to metabolize quickly. The use of drug carriers are needed to hold the drug at the site of treatment and increase the time of drug exposure to cancer cells[38]. Cisplatin itself should be prepared right before the procedure and use sterile sesame oil as the carrier[4]. Another method of incorporating cisplatin into treatment is through slow-release cisplatin-containing biodegradable beads[38]. These beads are 3 mm diameter in size and hold 1.6 mg of cisplatin or 4.6 mg of carboplatin[22]. The beads are surgically inserted subcutaneously next to a cutaneous melanoma nodule that is greater than 1.5 cm[22]. Treatment with cisplatin has been shown to have an 87.5% success rate of either eliminating or reducing recurrence for multiple years in the horse, but has only shown improvements in a local setting and not in a systemic setting[22].

In relation to cisplatin, electrochemotherapy is a relatively new procedure that incorporates short-term tumor permeabilization with chemotherapeutic agents[22]. During this procedure, electrical pulses are applied to selected tumors. The pulses allow increased cell membrane permeability to antitumor hydrophilic drugs, such as cisplatin[38]. For uniform distribution, cisplatin injections are spaced evenly around tumors, or if this treatment is post-excision surgery, injected evenly around the scars[38]. The electrical pulses are delivered by a Cliniporator by means of invasive or non-invasive electrodes[22]. As electrochemotherapy allows easy movement into the cell, it also drives cytotoxic agents into endothelial and stromal cells, which cause the blood supply and structure of the treated tumor to be disrupted[22]. Electrochemotherapy is performed with the horse under general anesthesia or sedation, and requires multiple sessions with standard 2 week intervals[22]. The effectiveness of electrochemotherapy is unproven, but it might be a cost-efficient option for control of equine melanomas.

Similar to electrochemotherapy, radiation therapy directly kills tumor cells with high-energy beams such as protons[17]. It is used commonly in humans and small animals, but has limited applications in equine medicine[22]. Most commonly, irradiation is used for small tumors in and around the eyes[22]. The delivery of radiation therapy in horses is highly specialized and often expensive. Cases have shown that this treatment option is not effective, does not reduce the size of the tumors, and does not improve recurrence or overall survival which means it is a treatment that is not used regularly for gray horse melanoma[22].

Immunological therapy, including biologic response modifiers, is an increasing field in cancer treatments across species. Cimetidine is a histamine-2 receptor antagonist used in both humans and animals to decrease stomach acid and prevent gastric ulcers [4]. The drug blocks the activation of suppressor T-cells in the microenvironment surrounding the melanoma[4]. Suppressor T-cells, activated by histamine, usually prevent activation of the immune system, dampening the natural response to neoplastic transformation, and allowing melanomas to persist within the body[14]. Blocking the histamine activation of suppressor T-cells with cimetidine enhances the body's ability to mount a cell-mediated and humoral immune response[4][14]. One study evaluated the use of cimetidine in 3 horses with dermal melanomas. Following treatment with cimetidine (2.5 mg/kg every 8 hours) for 2 -12 months, the melanomas decreased in both size and number in all horses and response was durable in two of the horses for at least 41 months[14]. Cimetidine was well-tolerated and in this small study appears to have some efficacy[4].

Lastly, treatment with xenogeneic DNA vaccines have shown promise. In a recent study, 3 groups of 9 gray horses were vaccinated on days 1, 22, and 78 with DNA vectors encoding for equine (eq) IL-12 and IL-18 alone or in combination with either human glycoprotein (hgp) 100 or human tyrosinase (htyr)[23]. The injection site for the vaccination on each horse was performed intramuscularly. One dermal melanoma was also treated individually by intradermal peritumoral injection. By singling out an individual melanoma on each horse, the researchers were then able to see the difference in size over time to see if having eqIL-12 and IL-18 by itself is better than in combination with either hgp100 or human tyrosinase or vice versa. Monitoring of the melanomas

concluded that all three options, the two combination variations and eqIL-12 and 18, reduced the melanoma volume significantly, 79.1% , +/- 26.1%, by day 120[23]. Thus, potentially all three vaccine formulations could be effective in the treatment of gray horse melanoma.

Canine Melanoma:

Background:

Canine melanoma is also relatively common, although unlike gray horse melanoma, it has been more widely researched as a translational model for human melanoma. Canine melanoma typically develops in older dogs, and has no sex predilection, but there are certain breeds that are prone to its development[32]. Common breeds affected include those with a darker haircoat and/or gums such as: Airedale Terrier, Boston Terrier, Boxer, Chihuahua, Chow Chow, Cocker Spaniel, Doberman Pinscher, English Springer Spaniel, Golden Retriever, Irish Setter, Miniature Schnauzer, Scottish Terrier, Poodles, Beauce Shepherds, Rottweilers, and Labrador Retrievers[37]. Canine melanoma arises in three distinct clinical manifestations based on anatomical location: cutaneous, subungual, and oral including buccal mucosa, and lips[37]. Melanoma accounts for 7% of all malignant tumors, occurring most frequently in the oral cavity, GI tract, central nervous system, nail bed, footpad, and eyes[32]. Tumors in the oral cavity are aggressive, with high rates of local recurrence following surgery, and a high metastatic potential to the regional lymph nodes and lungs[37], while cutaneous melanomas are typically more benign[32].

Clinical Presentation and Diagnosis:

Cutaneous melanomas typically present as small masses that are different shades of brown to black, however, the masses can also appear as large, flat, and/or wrinkled tumors that can be either slightly or very heavily pigmented[32]. Infrequently, the patient can develop a mass that will not have melanin, an amelanotic melanoma, making it more difficult to diagnose[32]. Clinical signs of an oral melanoma include difficulty swallowing, bad breath, increased saliva production, and bleeding from the gums and other oral tissue[32]. Frequently, the patient will develop an oral melanoma that invades the surrounding bone, which can fracture[32].

Treatment:

The best treatment option for any type of canine melanoma is surgical removal with wide margins. This is typically more feasible for cutaneous melanomas compared to oral melanomas. If surgery is not an option, or the risk for metastatic disease is high, alternative or additional therapies are needed including targeted therapies, chemotherapy, and DNA vaccines.

Classic treatment options for canine melanomas include chemotherapy (carboplatin), the melanoma vaccine, or non-steroidals. Although used frequently, chemotherapy has not been shown to increase survival times in dogs with melanoma[27]. A study done by L. K. Brockly evaluated 63 dogs with oral, digital or cutaneous malignant melanoma to investigate if chemotherapy significantly impacted outcome. Unfortunately, there wasn't a large increase in

survival rate for dogs receiving chemotherapy. However, the literature is mixed, and some individual dogs had excellent responses to chemotherapy. Thus, it is still offered as a standard of care alone or in combination with surgery.

The Oncept canine melanoma vaccine is another treatment option. This is a DNA-based vaccine, spliced with human tyrosinase, to stimulate the canine immune system against the malignant melanoma cells[9]. A study by Ottnod evaluated the medical records from 45 dogs with stage II or III oral malignant melanoma over 12 years. Dogs were separated into 2 groups: those who received the vaccine (n = 22) and those who did not (n = 23). Breed, sex, and age of the patients were similar in both groups, along with the median year of initial diagnosis[26]. Results from this study indicated there was no therapeutic advantage following use of the vaccine for any of the stages of oral malignant melanoma, as there was no impact on overall survival time or disease free interval. This could be due to the lack of a consistent schedule for the vaccinations and rechecks, their minimal use of CT scans or necropsy, clinical biases, the diversity of surgical approaches prior to treatment, or even the use of additional therapies[26]. Other studies have suggested that Oncept does confer a therapeutic advantage, so the literature is mixed and additional, prospective studies are needed[13].

There is evidence that canine malignant melanoma cells increase cyclooxygenase-2 (COX-2), which catalyzes the transformation of arachidonic acid to prostaglandin[3]. COX-2 drives inflammation and increases invasiveness of tumor cells and anti-apoptotic effects, protecting tumor cells[34]. The creation of specific and selective COX-2 inhibitors (celecoxib) has shown inhibitory effects on cell growth in different human cancers such as malignant melanoma[34]. The study done by Seo looked at how celecoxib affected COX-2 high-expressing areas in canine malignant melanoma cell lines. The cell lines were LMeC, coming from canine oral mucosa, and CMeC-1, coming from the skin[34]. COX-2 was found to be highly abundant in LMeC cells but not in CMeC-1 cells, and treatment with celecoxib for 48 hours decreased the amount of COX-2 in the LMeC cells most substantially[34]. This study concluded that treatment with celecoxib did suppress the growth and proliferation, and caused tumor cell apoptosis, in both canine malignant cell lines, but had a greater effect in the cell line with more COX-2 expression.

An emerging treatment option includes the use of oral tyrosine kinase inhibitors. Receptor tyrosine kinases (RTKs) are a group of surface proteins on a cell that play an important role in cell growth and proliferation[6]. Following binding of a growth factor from the microenvironment, the receptor is activated, sending signals through the cell to grow and divide. Tyrosine kinase is a protein that phosphorylates other proteins with its tyrosine residues[16]. An example of a RTK is epidermal growth factor receptor (EGFR). If this receptor binds to a growth factor, then two RTKs will dimerize and activate each other with the assistance of ATP[6]. A kinase cascade that stimulates other membrane proteins follows the initial dimerization and will eventually lead to gene transcription and expression[6]. Tyrosine kinase inhibitors prevent the intracellular signals, which then in turn alter cell growth and proliferation[30]. These inhibitors

decrease or stop the infected cell from growing and dividing, which also inhibits its ability to metastasize[30].

The only available tyrosine kinase inhibitor in veterinary medicine is called toceranib phosphate, or Palladia. Palladia received FDA approval for the treatment of canine cutaneous mast cell tumors[18] but has been shown to have activity against multiple tumor types[19]. Individual cases have suggested that Palladia might have a biological effect against canine melanoma[43][19].

Definitive treatment recommendations for canine malignant melanoma are lacking, due to the mixed results presented above. More studies are needed to determine which treatment modality will increase the survival time and have the greatest impact for canine patients with melanoma.

It is thought that canine cancers, specifically melanoma, develop due to genetic factors, following exposure to environmental and chemical carcinogens, exposure to radiation, and hormonal changes, similar to cancer-causing effects in people[25][37]. In people, a major cause of cutaneous melanoma is exposure to UV light radiation, which appears to be the case in some canine melanomas as well, making dogs an excellent translational model for humans.

Human Melanoma:

Background and Genetic Analysis:

Human melanoma is one of the most devastating types of cancer, killing ~50,000 to 55,000 people over the world every year[25]. In a study from 1982 to 2011, the increase of melanoma around the world increased an average of 3%. In more specific areas of the world, predominantly in the US and Australia/New Zealand, there is an increased risk of melanoma due to skin type, age, and extended time under the influence of UV radiation (UVR)[33]. An increase in people developing this disease is projected to continue in these areas until 2022, although Australia is one of the few countries decreasing their overall cases of cutaneous melanoma due to their increased efforts of prevention and lifestyle changes[33].

Malignant cutaneous melanoma in humans is the most aggressive[25]. It is resistant to therapy and is a deadly form of skin cancer[25]. Family history plays a major role in its development: humans of European descent are more susceptible to CSD (cumulative sun-induced damage) or non-CSD melanoma compared to those of Asian or African descent[32]. Other risk factors include skin and mucosal pigmentation, mole irregularities, and sun exposure, notably to UVB light, and how the skin reacts to such exposure[32]. Another form of melanoma that is also aggressive, but rare, is oral malignant melanoma[25]. This is similar to canine oral malignant melanoma, and this form of neoplasm in humans is usually seen in middle-aged adults[25].

As previously mentioned, UVR plays a significant role in the development of cutaneous melanoma in people, especially those of Caucasian descent. It has been shown that having 5 or more sunburns increases the likelihood of developing melanoma[44]. Another study that investigated a set of case‐controlled studies of melanoma found that intermittent exposure and

sunburn in adolescence or childhood were strongly correlated with greater risk of melanoma[1]. Despite the evidence that UVR exposure impacts the development of cutaneous melanoma in Caucasians, it is more nuanced in people with darker skin tone.[1]. Those of ethnic descent often develop melanomas on the sole of the foot, the palm, subungual, and other mucosal surfaces that are protected from the sun[1]. Looking at how sun exposure affects the melanocytes could shed some insight on why these discrepancies exist.

It is thought that carcinogenic, inflammatory, and immunosuppressive properties of UVR all contribute to initiation, progression, and metastasis of primary melanoma[12]. UVR has three components: UVA, UVB, and UVC[5]. UVA makes up 95% of UVR ranging from 315-400 nm and UVB making up the remaining 5% of solar UVR and ranging from 280-315 nm[45]. Even though UVB has a less percentage makeup of the total effect of solar UVR, it is what's most powerful in generating cancer in animals, creating sunburns in humans, and also causing more DNA damage than UVA[45]. UVC does not reach the earth's surface like UVA and UVB due to the atmosphere around earth absorbing this range of radiation[45]. The impact of UVR is modulated by the environment. Factors that influence the amount of UVR reaching people on the earth's surface include: time of day, latitude, altitude, weather conditions, and reflections[45]. The time of day when the sun is at its strongest is the halfway point between sunrise and sunset[45]. The equator receives the strongest UVR, while it is lowest in higher latitudes as the sun sits lower in the sky[45]. Higher altitudes also have strong levels of UVR. Weather conditions, such as heavy cloud coverage protects from the sun's radiation, but not completely[45]. Lastly, the sunlight is able to reflect off of many things to intensify its radiation. Water, snow, and sand are able to reflect up to 90% of the original UVR[45]. Thus, many environmental factors increase the chances of developing melanoma.

UVR exposure causes DNA damage. As previously presented in gray horse melanoma, the MC1R pathway along with the cAMP pathway in humans is disrupted when UVR alters DNA[11]. In gray horse melanomas, the MC1R pathway is a major component of skin and hair pigmentation and is already highly susceptible to polymorphisms before additional damage can be done by UVR[11]. The research done by García-Baron suggests the MC1R pathway plays a similar role in humans. MC1R is part of the biggest families of surface cell receptors in the human genome and is in a subfamily of G-protein channel receptors[11]. Once MC1R is stimulated by an endogenous agonist, a cAMP signalling cascade occurs[11]. This stimulated cascade then leads to the increase of tyrosine, which increases the production of eumelanin[11]. UVR affects MC1R protein sequences by creating multiple polymorphisms[11]. These polymorphisms have specific mutant amino acids and those amino acids are what trigger alterations in programming other proteins for cell functions[11]. The alterations transform the genes that deactivate MC1R altogether and ceases MC1R's ability to repair UV-induced DNA damage and eumelanin production[11]. This is also seen in gray horses, potentially explaining their high probability of developing melanoma. The ASIP mutation can also occur in humans, and similarly to gray horses, it will prevent eumelanin production[11]. In humans, ASIP has

modulation powers over multiple genes and has involvement in redox metabolism, cell adhesion, and other cellular processes other than pigmentation[11].

Clinical Presentation and Diagnosis:

The specific clinical presentation of melanoma is not the same for each individual as it depends on location on the body and type of growth[36]. There are four main histopathological types of melanoma: nodular, lentigo maligna, acral lentiginous, and superficial[36]. Irregularities that could indicate melanoma development are identified using ABCDEFG criteria:

A – Asymmetry; one half of a nevi or birthmark does not match the other[41].

B – Border; the edges are irregular, jagged, or blurred[41].

C – Color; the color is not the same all over and may include shades of brown or black, or sometimes with patches of pink, red, white, or blue[41].

D – Diameter; the spot is larger than 6 mm across, although melanomas can sometimes be smaller than this[41].

E – Evolving; the nevi are changing in size, shape, or color[41].

F – Feeling; rough in touch[36].

G – Growth; increasing in size over a period of time (months or years)[36].

Another useful diagnostic tool to recognize the development of melanoma is called the "Ugly Duckling sign"[36]. This approach is based on observations of each person with multiple nevi (moles) having a specific profile[36]. As a result, if there is a lesion that looks unusual compared to the surrounding lesions, it should be considered suspicious (Ie. the "ugly duckling) and be more closely examined or excised is necessary[36]. Once a correct diagnosis has been made, there are multiple options for treatment.

Treatment:

Treatment approaches for human melanoma are similar to gray horse and canine treatments, including cimetidine, tyrosine kinase inhibitors, chemotherapy, and combination therapy with BRAF-MEK inhibitors. Use of cimetidine, chemotherapy, and tyrosine kinase inhibitors are implemented in similar ways in each species. In humans, a new treatment option is pembrolizumab, an anti-programmed cell death protein-1 (PD-1) humanized monoclonal antibody[24]. Pembrolizumab blocks PD-1 and its receptors, PD-L1 and PD-L2, allowing activation of T-cells to locate infected cells and either create an immune response or kill the infected cells[24]. It displays the second breakthrough in immune checkpoint blockade therapy for melanoma[42]. The study mentioned by Weide concluded that pembrolizumab was active against many different kinds of solid tumors and increased the overall survival time for melanoma patients.

Combination therapy with BRAF-MEK inhibitors also shows promise in the treatment of human melanoma. BRAF is often mutated in human cancers, especially human melanoma [10].

BRAF-MEK inhibitors block activity coming from the mutation[10]. The results from a review by Eroglu showed an increase in response rates, up to 70%, with combination BRAF-MEK therapy and that it may be effective in patients even without a BRAF-MEK mutation. Combination treatment with anti-PD-1/PD-L1/2 and BRAF-MEK inhibitors may be possible and may increase response to treatment. Although there are several promising treatment approaches for human melanoma, additional studies and treatment alternatives are needed to improve the overall outcome for those afflicted by this tumor. The five year prognosis depends on the thickness of the primary tumor, if the lymph nodes were involved, and whether or not it metastasized to distant parts of the body[15]. If the melanoma did not spread to lymph nodes or distant areas, the five year survival is 99%[15]. In contrast, those who have a thicker melanoma, the prognosis decreases to 80% and if it has spread to local lymph nodes, the percentage decreases to 65%[15]. These numbers reflect averages and not for each specific individual and so the prognosis could be less than 65% or better depending on the individual immune system capability to work against the melanoma.

Conclusion:

Skin cancer can develop in multiple species, and although developmentally different, treatment of gray horse, canine, and human melanoma are similar. In gray horses, a duplication mutation in STX17 leads to increased proliferation of melanocytic cells, leading to melanoma development in some individuals. However, many gray horses that develop equine melanoma are genetically predisposed, with the gray coat color and propensity for melanoma being genetically linked. Canine melanoma can occur due to genetic predispositions, exposure to sunlight, and other environmental factors, much like humans. The main cause of human melanoma is exposure to intense sunlight, especially in people of European descent with fair skin. In all three species, cutaneous melanoma is malignant, and can be aggressive and difficult to treat. Particularly in humans and dogs, effective treatment options are lacking.

In all three species, surgical removal is the first treatment of choice. Although this is often delayed in gray horses unless the tumor is causing physical discomfort, as in this species cutaneous melanoma appears to be less aggressive. Additional treatment options include injectable chemotherapy, oral cimetidine, and immunotherapy, although a positive impact on overall survival time has not been definitively proven for either modality. Cryotherapy following surgery has shown the potential to increase the progression-free interval in humans and gray horses. Canine melanoma has been shown to have increased levels of COX-2, and treatment with a COX-2 inhibitor has anecdotally been suggested to result in therapeutic response. This is a potential avenue for investigation in human and gray horse melanoma.

Cutaneous melanomas in gray horses, canines, and humans have more in common than thought previously, but they all need more research to establish effective treatment protocols. Given the similarities between the species, many of the results found in one species may be translated to another. There are some very promising treatment options for melanoma, but

continued research is necessary to find the root causes of this disease and improve the outcome for gray horses, canines, and humans diagnosed with cutaneous melanoma.

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