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Perspective

A Worldwide Preventative Cancer Vaccine Is Achievable With New Discoveries And Comparative Oncology

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Abstract

Cancer remains the leading cause of death worldwide, even in the face of enormous research and clinical efforts. These have yielded some promising therapies; however, they are neither broadly effective nor affordable. One of the most valuable new treatment classes has been immunotherapies, including vaccines. The different types of cancer vaccines and their characteristics, along with their contributions and shortfalls are summarized here. A more effective and significantly less expensive alternative to personalized therapeutic vaccines is proposed here to be an off-the-shelf, prophylactic one. However, knowing what antigens a future tumor will present has been considered impossible. The discoveries and technologies that together enable the identification of these types of vaccine components are described. A pre-made, preventative cancer vaccine has recently shown efficacy in a canine clinical trial. The path is opening for the development of effective vaccines to prevent cancers in people worldwide.

Keywords: Preventative Vaccine; Multi-cancer vaccine; Neoantigens; Comparative oncology; Worldwide market; Antibody biomarkers.

Introduction

Medical and economic impact of cancer

The physical and emotional costs of cancer cannot be quantified; however, incidence and death can. In 2023, nearly 2MM new cancer cases will be diagnosed and over 600K human deaths will occur in the US alone [1]. Cancer is the leading cause of mortality worldwide, claiming a tally of 10MM lives annually [2]. Using a macroeconomic model, the global cost of cancer from 2020-2050 is estimated to be over $25 trillion, covering medical interventions and care. Although 75% of the cancer deaths occur in low and middle income countries (LMIC), the burden of treatment costs is greatest in higher income countries [2]. This is driven by the high and increasing costs of therapeutic regimens, and their consequent inaccessibility to LMIC.

On a positive note, the higher costs are predominantly a result of newly available treatments. Therapies have extended beyond conventional surgery, chemo- and radiation-therapy to now include options such as immune-, targeted-, stem cell-, nanoparticle-, sonodynamic-therapy and others [3]. These new treatments are important advancements and have saved lives. However, a therapeutic is defined by being provided in response to a disease or disorder. An alternative is prophylaxis, providing a product to prevent the disease from happening. Medics have successfully undertaken this approach to prevent infectious diseases using vaccines for over 200 years. Yet cancer, a non-infectious disease of self-cells gone awry, is not generally considered addressable in the same manner. What if it were? Preventative vaccines, as evidenced by infectious disease examples, are the most cost-effective medical intervention ever discovered. If an effective pan cancer preventative vaccine were developed, the medicine and economics of cancer would be transformed, healthcare costs would plumet and all people of the world would have access to cancer prevention. A preventative vaccine means that symptoms are not suffered. There are no patients.

First dogs, then people

The sharing of medical treatments between people and animals is certainly nothing new. Many veterinary treatments begin as human ones. Research and development costs that are affordable relative to a human market are often not supportable for the animal ones. However, once a human therapeutic is developed, animal applications become possible. Cross-over use is common for a myriad of drugs and indications. For instance, gabapentin, tramadol and pregabalin are commonly prescribed pain medications for dogs and horses. Narcotics such as fentanyl, morphine and ketamine, the antibiotic amoxicillin in addition to several chemotherapy drugs developed and approved for human use are also commonly prescribed for pets. The list is long (drugtopics.com, vettechprep.com). The therapies can also transition in the other direction. Unsanctioned but nonetheless common, is the use of pet therapeutics in humans. For instance, veterinary antibiotics are more accessible and less expensive than their human counterparts [4]. In addition to shared drugs, there are vaccines that are shared between dogs and people. For example, the rabies vaccine is fully protective against infections in both hosts and works both therapeutically and prophylactically [5,6]. A preventative Rift Valley Fever vaccine has been co-developed for both human and livestock species [7].

At the molecular level, studies have demonstrated that dogs closely mirror human disease profiles of cancer and other chronic diseases [8]. Clinically, dogs spontaneously develop cancers that frequently have significant similarity to those that arise in people, and the use of dogs with cancer to assist in development of anti-cancer therapeutics is becoming more common [9-11]. The study of naturally developing cancers in animals as models for human disease has become known as comparative oncology. It's popularity has grown since the dedication by the National Cancer Institute of a large research program in 2003 called the Comparative Oncology Program (COP) (https://ccr.cancer.gov/comparative-oncology-program). As tumors in dogs arise in a similar manner and with similar host-immune context as those in humans, dogs are an opportune population for evaluations of immunotherapies, including vaccines, being developed for people [11]. Additionally, the shorter lifespans of dogs and their accelerated courses of tumor progression versus that of humans allows for more compressed trial timelines of safety, immunogenicity, and efficacy.

In another relation, the regulatory paths for developing medical products in companion animals and people are similar, both emphasizing safety and efficacy. However, the USDA trials can be less complex, such as requiring only two phases instead of the familiar three (or four) FDA phases. One key trial design difference is the USDA's focus on testing a veterinary intervention in the target animal. By contrast, human trials typically begin with pre-clinical studies in laboratory animals and then progress to the human target species. This distinction underscores the relevance of non-human results in the FDA's approval considerations.

We suggest using comparative oncology as a means of accelerating the development and commercialization of preventative vaccine products against cancer for people. Companion animals, such as dogs, are not only models to facilitate the study of human disease. They also represent a real and significant business market for oncology products, both in size and value. Furthermore, products that are price-appropriate for animal healthcare would make them accessible beyond high-income countries. The availability of an affordable pan-cancer vaccine that protects dogs may help convince the community that a human product is also possible. Will this drive its development and commercialization worldwide?

Characteristics of existing cancer vaccines

Conventional categories of tumor antigens

All existing cancer vaccines, both human and canine, are therapeutic. The first human vaccine was based on lysates of a colorectal patient's resected tumor [12]. The same approach, an autologous, personalized tumor cell-based vaccine for resectable solid tumors, has more recently been developed in dogs [13] and trials are being conducted for USDA approval (Torigen Pharmaceuticals and Ardent). Further research efforts in this area led to therapeutic vaccine designs that begin with surgical excision of a patient's tumor and then use of the lysate, exosomes or RNA as a source of undefined antigens to load autologous antigen-presenting-cells that are expanded *in vitro*. Adjuvant is added to the antigen mixture and then re-injected into the same patient [14]. Currently, the only FDA-approved anti-tumor vaccine is Sipuleucel-T (Provenge), an autologous, dendritic-cell based composition of unknown antigens for treating metastatic prostate cancer [15]. When it was released in 2010 at $93,000 per regimen, there was a media outcry about the high price tag [16]. Even more unfortunate, the subsequent finding that the product provides only marginal survival advantage led to a general disenchantment with all cancer vaccine efforts [17].

The identification of tumor-associated antigens (TAAs) [18] launched a large body of studies using these normal proteins that are inappropriately expressed in tumor cells such as human melanoma-associated antigen 1 (MAGE-A1), human epidermal growth factor receptor 2 (HER-2) and prostate-specific antigen (PSA) among others. The over-expression of tyrosinase on melanoma cells was used to develop the canine melanoma vaccine product Oncept (Boehhringer Ingelheim). This DNA vaccine is a plasmid expressing human tyrosinase (TYR), which is sufficiently foreign in dogs to raise an immune response, yet

similar enough for anti-TYR immune cells to recognize the tumor and slow recurrence [19]. However, this class of antigens in cancer vaccines, even as adjunctive treatment, has generally met with limited success. The disappointing results have been attributed to their weak immunogenicity. As self-antigens they display little to no foreignness to the immune system, and therefore may not break central tolerance. Expression of TAAs in normal host tissues also increases the risk of autoimmune toxicity [20].

A new class of tumor antigens: neoantigens

In contrast to TAAs, tumor-specific antigens (TSAs), more recently referred to as neoantigens, are only expressed by tumor cells, or at least only presented to the immune system in tumor cells. As non-self, they are more likely to trigger anti-tumor immune responses, and not cause autoimmunity. Recent approaches have focused on using tumor neoantigens to develop personalized, therapeutic cancer vaccines [21-23]. These neoantigens are the result of mistakes in DNA replication, predominantly somatic point mutations in coding regions or mutations from genomic instability at microsatellite loci. In healthy cells, these errors are very rare, a biological consequence of the highly evolved DNA proofreading and repair processes that maintain the integrity of a cell's genome [24]. In tumor cells, mutation rates become higher as genome maintenance is loosened to support rapid proliferation, although mutations are still relatively rare. To identify these mutations, tumor samples are obtained, DNA is extracted and then sequenced. A great deal of data is now available showing that these are largely tumor and patient specific, therefore necessitating a vaccine to be personally designed and produced for each patient. Even after a patient's sequencing data is established, the mutations that will be transcribed and translated remain unknown. Point mutations will encode aberrant proteins with only single amino acid changes (if not silent), and therefore will typically be weak antigens. Since immunogenicity is both unknown and likely to be low, bioinformatic algorithms are employed to improve the probabilities of selecting mutations that will encode antigens with CD4+ and CD8+ T cell recognition. An alternative approach to identifying expressed and immunogenic mutations has been mass spectrometry analysis of MHC-bound peptides removed from the surface of dendritic cells. However, limited levels of neoantigen peptides on the cell surface and limited amounts of patient tissue have compromised the sensitivity and accuracy of these methods [25].

Over the last few years, clinical trials have been conducted to evaluate these personalized, therapeutic neoantigen vaccines. This individualized approach has provided some marginal reductions in tumor activity in patients with melanoma and other cancers [26,27]. They require a resectable tumor, DNA sequencing, several months of lab manufacture and >$100,000 to build. As these vaccines are typically administered to patients with late-stage disease, the months required for their preparation can be too long. Tumor escape and logistical obstacles to vaccine design remain additional challenges [28].

More encouragingly, the results of two personal neoantigen vaccine trials were recently reported that demonstrated clear benefit, one for melanoma [29] and one for pancreatic cancer [30]. Larger clinical trials are currently underway (BioNTech.com, Modernatx.com). However, responses to the vaccines required the co-administration of a check point inhibitor. The combination of the vaccine plus checkpoint inhibitor would double the treatment cost, further restricting patient accessibility. These factors combined with our economic era of clearly limited resources and the widespread concern over rising health care costs, have led to debates on the value of therapeutic cancer vaccines and other immunotherapeutic interventions. Despite this negative environment, the potential impact of a successful cancer vaccine has driven exploration of alternative approaches.

Characteristics of a preventative cancer vaccine approach

Prophylactic advantages

Mounting an effective immune response to an existing tumor is significantly more demanding than preventing one for several reasons. For instance, a 1 cm^3 tumor is typically comprised of a billion tumor cells [31]. Even against such a relatively small-sized, isolated mass, immune effectors need to attack against a superior force. The numbers become more overwhelming as disease progresses. Furthermore, when tumor cells initially arise, they are exposed to a functioning immune system. However, as the tumor develops, a microenvironment co-develops to thwart anti-tumor immune activities. The tumor microenvironment (TME) is a complex and continuously evolving entity of molecular, cellular and physical changes to host tissues that supports disease progression [32]. Beyond the localized TME, there is also a myriad of systemic mechanisms that induce host changes and functionally compromise individuals' responses to their established disease [33]. By contrast, setting up an immune response to attack a small number of emergent, unprotected tumor cells in an individual with an intact immune system would logically have a higher likelihood of success. Analogously, infectious disease vaccines are only effective if administered before the infection occurs.

Few efforts have been directed to cancer prevention beyond lifestyle changes such as smoking cessation, dietary changes, or exercise. Preventative vaccines have not been widely investigated due to the observation that tumor mutations are patient and tumor specific. With this characteristic, anticipating an antigen for an individual before the tumor exists is problematic. Uniquely, two cancer types carry mutations that are shared across tumors and known prior to disease. This has enabled development of

preventative vaccines for these atypical cancers. First are those caused by a viral infection [34,35]. Human papilloma virus (HPV) causes cervical, anal, vulvar, vaginal cancers; hepatitis B virus (HBV) causes liver cancer [36]. Preventative vaccination strategies have been very successful in reducing their incidence. Although, these are arguably not cancer vaccines since the vaccine protects the host from the virus, not formally the tumor. The second type is cancers associated with Lynch syndrome, an inherited deficiency caused by a few predictable DNA frameshift mutations in a defined set of mismatch repair genes [37]. Trial enrollment recently started to evaluate a vaccine comprised of DNA mutation-derived antigens to prevent or delay cancer onset in individuals with Lynch syndrome (https://classic.clinicaltrials.gov/ct2/show/NCT05078866). If successful, this trial will demonstrate that a vaccine can directly prevent cancer, if protective tumor-specific antigens can be anticipated and administered before tumorigenesis.

Prophylactic challenges

Aside from these few exceptional cancers, a preventative vaccine against cancers has been perceived by many as an unreasonable goal. Causes for skepticism include the inherent requirements of anticipating neoantigens that will be made by future tumors of unknown types. Mutations would need to be commonly made among different tumors and tumor types, expressed by the tumors, and ultimately elicit long-lasting, protective immune responses in each patient within a large, diverse population.

The feasibility of anticipating immunogenic tumor antigens for preventative cancer vaccines

Shared, tumor RNA-error-derived-neoantigens (REDNs)

Unlike DNA synthesis, cells make unchecked, molecular mistakes during and following RNA synthesis. The generation of mRNA and its translation involve hundreds of proteins and many cellular processes including transcription, editing, exon splicing, capping, polyadenylation, mRNA transport, initiation of translation and mRNA turnover. In all cells, errors in RNA transcription and processing occur more than 100-fold more frequently than errors in DNA replication [38]. RNA error rates in tumors are further escalated relative to healthy host cells. Many RNA mis-processing steps, such as exon-skipping or intron retention during splicing, can generate transcripts with shifted coding frames. If translated, these transcripts will create proteins with a string of incorrect amino acid sequences at their C-termini. Normal quality control pathways that target aberrant RNAs for degradation [39] are both impaired and overwhelmed. Protein quality control systems such as the ubiquitin-proteosome pathway and autophagy [40] are similarly malfunctioning. Consequently, many aberrant RNAs are generated by tumor cells and translated into peptide variants. As many of these variants are frameshifts, they are more foreign than single amino substitutions and highly likely to elicit both T and B cell responses. This cache of tumor-cell specific aberrant RNAs is intuitively valuable as an alternative to the limited number of genomic mutations as a source of encoded neoantigens. The sheer number of errors suggests that the same errors might occur repeatedly, for instance in different patients and diverse tumor types.

A variety of approaches can be considered for searching tumor RNA for errors that encode frameshifted peptides. The earliest efforts were conventional molecular biology-based techniques prior to the widespread availability of RNA-sequencing. In one study, approximately 500 splice junctions catalogued in EST library databases were queried by RT-PCR against RNA samples extracted from a mouse melanoma cell line. Cloning and sequencing of the PCR products identified 3 different mis-spliced junctions, which would encode frameshift peptide variants. The frameshift peptides were synthesized and determined to be immunogenic. The pool of peptides displayed anti-tumor effects as vaccines in mouse models of melanoma and breast cancer [41]. While this preclinical work was successful in demonstrating the utility of the RNA-error derived neoantigens (REDNs), the method is very labor- and time-intensive and provided the identification of only a small number of neoantigens. Alternatively, computational approaches can be enlisted to detect mis-processing events from tumor RNA-Seq data. Among the challenges to this method are the low accuracy of RNA-sequencing and the requirement for an informative read to span the mis-processing event. For example, aberrant exon/intron junctions would need to be captured to establish the new coding frames created from intron retention or exon skipping. Nonetheless, researchers have shown that a number of variants can be identified in cancer patients with predicted HLA-binding affinity [42]. Recently, RNA-Seq databases have been purposed as means of verifying DNA-seq results and indicating which DNA mutations are transcribed in a tumor [43,44]. While useful for neoantigen discovery based on DNA mutations, this approach does not search the RNA error derived neoantigen source. Any transcriptional-level error will not have a genomic reference and thereby be dismissed. In contrast, Tretter et.al. conducted a comprehensive analysis including genomics, transcriptomics, proteomics, artificial intelligence and immunomics of 32 cancer patients to identify 21 tumor neoantigen candidates [45]. They revealed that variants are more far more common at the RNA-level than DNA-level and that some are shared between different tumors.

Taken collectively, these efforts confirm that RNA dysregulation in tumors is an important source of RNA variants that would encode peptide variants. Some are shared across tumors and tumor types, and some of these may function as tumor neoantigens. Sharedness is an essential characteristic of the

antigens to comprise a successful preventative vaccine. If a set of neoantigens were identified that collectively represented those made by all tumors and were immunogenic in all patients, then a prescient vaccine could be prepared for healthy people, before tumorigenesis. However, the challenge is developing a method to sift through a sufficiently large body of the RNA variants to find those that are broadly, frequently shared and immunogenic. A highly scalable, accurate, and sensitive methodology is required for searching this massive RNA sequence space for frameshift-generating errors that will stimulate anti-tumor immune responses capable of providing protection for everyone.

A screening technology for identifying shared, tumor-REDNs

The criteria of efficient, accurate, and sensitive can be used to evaluate methodologies for discovering which RNA errors will encode optimal, preventative vaccine components. The size of the RNA sequence space to be searched demands a high throughput approach for efficiency. At the RNA level, there remain many physiologically unknowns about the outcome of any RNA-error. The probability of accurate identification of an immunogenic REDN would be improved if screening is not done at the RNA level, but rather done directly on an immune response that the REDN may have stimulated. Sensitivity of the screen will depend on the biomarker used for detecting the presence of REDNs in tumors, and their absence in host cells. While perhaps not the only solution, we have developed a technology that meets these specifications.

Nearly all possible RNA synthesis and processing errors can be informatically predicted, along with their encoded frameshift peptides. These variant peptides can be synthesized *in situ* on silicon wafers as high-density peptide arrays. These microchips provide a comprehensive display of putative REDNs that may, or may not, be expressed and immunogenic in a cancer patient. To identify useful REDNs, serum-antibodies from patients diagnosed with early disease are applied to the peptide-ligand arrays. Specific antibody binding is detected in a workflow resembling an ELISA, though conducted under highly-parallel, competitive and stringent conditions. Since antibodies are immune effector molecules, immunogenicity of the bound peptide is established. Since activated B cells proliferate, mature, and massively secrete antigen-specific antibodies, antibodies are highly sensitive biomarkers. This antibody-based microchip technology enables the screening of large numbers of patient sera for antibodies that bind REDN-peptides. Those REDN-peptides that are commonly bound by samples collected from many different cancer patients, and not healthy individuals, are strong candidates for inclusion in an off-the-shelf, preventative vaccine.

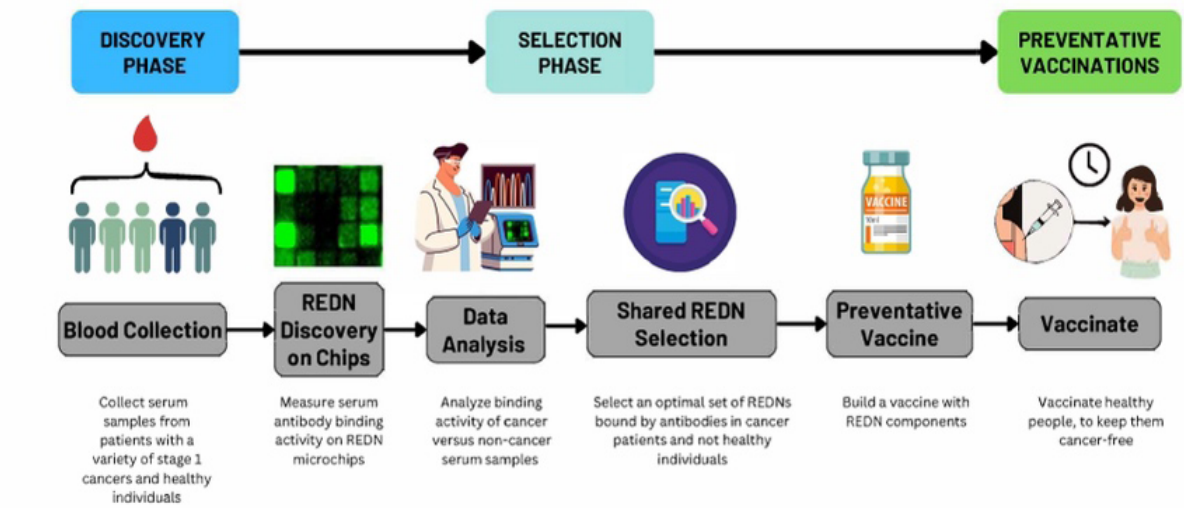

Figure 1: Development steps to making a REDN-based preventative cancer vaccine.

Conducting a preventative cancer vaccine trial

Trial population

The design and execution of a REDN-vaccine efficacy trial should be consistent with the mission of delivering to market a human, protective, cancer vaccine as efficiently as possible. Human trials, as discussed, can be lengthy and expensive. Subsequent regulatory approval and market penetrance can also be lengthy and disappointing. As a solution, comparative oncology can be applied. Do it in dogs first. Dogs have somewhat similar body sizes and naturally develop spontaneous tumors at the same lifetime risk (30%) as people. Their tumors are very like human ones in terms of structure, immune environment, and clinical presentation [46]. Furthermore, canine cancers have similar genetic and molecular targets, and display similar disease progression profiles relative to their human counterparts. These points may justify an assumption that if a vaccine design works in dogs, it will work in humans, too (Figure 1).

An important distinction between the species is the timeline of disease, which is significantly shorter for dogs. This reflects their generally compressed lifespans compared to that of people. The median age of canine cancer onset is 8.8 years [47] versus a median age of 66 years for human cancer diagnosis (https://seer.cancer.gov/statfacts/html/all.html); time to death following diagnosis is more rapid for dogs as well [48]. Consequently, conducting a trial with a canine population will yield endpoint results much faster than possible with humans. Another advantage to selecting a canine population is the feasibility of initiating a trial with a sufficiently large cohort to accommodate both safety and efficacy at once.

Trial Design

We designed a REDN vaccine to prevent cancer in dogs and are evaluating it in a clinical trial called the Vaccination Against Canine Cancer Study, with 800 owner-enrolled dogs (VACCS) [49]. To enrich the study population with dogs more likely to develop cancer during the 5-year study, middle-aged (5.5-11yro) dogs were enrolled. Healthy pets without a previous or current cancer diagnosis were recruited into this randomized, double-blinded, placebo-controlled, prospective trial at three oncology centers. The objective of VACCS is to determine the safety and efficacy of the vaccine. The primary endpoint is the cumulative incidence of dogs developing malignant neoplasia of any type at the end of the study period. Secondary endpoints include assessing adverse effects, changes in incidence of specific tumor types, changes in survival times following neoplasia diagnosis, and all-cause mortality.

The vaccine for this trial is comprised of a microchip-selected set of REDNs in a prime-boost immunization regimen: DNA prime and peptide boost. In the vaccine arm, the DNA prime encodes 31 REDNs strung together on two nanoplasmids [50]. In the placebo arm, the DNA prime encodes an irrelevant peptide [51]. As a genetic adjuvant for the prime, a DNA plasmid encoding dog granulocyte-macrophage colony-stimulating factor (GM-CSF) is included in both vaccine and placebo. The vaccine arm peptide boost contains 20 of the 31 REDNs as synthetic peptides. The placebo arm boost contains the irrelevant peptide encoded by the placebo arm DNA plasmid. Hiltonol [52], a double-stranded RNA, is included as peptide adjuvant for the boost in both arms. Boosts for the initial immunization regimen are administered once a year. Sera and PBMC's are drawn for immune analyses. Clinical examinations are conducted every 6 months by a veterinary oncologist. An independent safety board reviews data each year; no vaccine related adverse events have been identified. The trial will be completed, unblinded, and assessed for efficacy in May 2024. Initial indications are promising.

Perspectives

If the availability of an effective, dog preventative vaccine can generate demand for a human product, then we should be prepared to deliver one. The same innovations and technologies that led to the discovery of the canine vaccine can be applied to discovering a human one.

First steps would involve acquiring serum samples from cohorts of patients diagnosed with different early-stage cancers. These would be assayed on human REDN microchips to select optimal, immunogenic neoantigens to comprise the vaccine. As with sample analysis on the dog REDN microchips, human REDNs would be selected that were bound by cancer patient sera and not bound by sera from healthy individuals. While these REDNs are obvious candidates for vaccine compositions, they can also collectively serve as biomarkers for early-stage cancer detection. We have demonstrated this application of the chips with sera from canine cohorts. In this diagnostic capacity, the microchips could facilitate the vaccine clinical trial. For instance, microchip testing of human sera could be used to confirm the cancer-free status of trial enrollment candidates. This should optimize data clarity because there would be no accidental inclusion of someone with existing cancer, though undetected by standard diagnostic evaluations. The eventuality of missing a tumor during enrollment would otherwise need to be accommodated by a larger trial population. Once enrolled and vaccinated, regular REDN diagnostic testing should detect early-stage disease, where conventional methods are less sensitive. This faster identification of disease onset would reduce trial timelines and costs.

Regulatory approval of a vaccine requires attention, time, money, and a strong data package. As described above, the dog preventative cancer vaccine results could be a significant part of

this dataset to include in an FDA application. Furthermore, once the human vaccine is approved, the existing market presence of a dog preventative vaccine may stimulate demand and facilitate distribution of the human product. If people have already vaccinated their dogs, they are familiar with this unconventional cancer product; hence, awareness and acceptance should be lower marketing hurdles. Finally, one of the reasons that the dog product described here can be pursued before a human one is that the underlying technology is inexpensive relative to current cancer therapeutics. Consequently, the market price can be much lower than other medical interventions. Worldwide availability becomes possible.

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Conflict of Interest

Kathryn Sykes and Stephen Johnston are employees of Calviri, Inc.

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