Veterinary Oncology

Elsevier Editorial System(tm) for The Veterinary Journal

Manuscript Draft

Manuscript Number:

Title: Veterinary Oncology: Biology, Big Data and Precision Medicine

Article Type: Commissioned Review Article

Keywords: cancer, canine, big data, precision medicine

Corresponding Author: Prof. David Argyle,

Corresponding Author's Institution: UNIVERSITY OF EDINBURGH

First Author: David Argyle

Order of Authors: David Argyle; Lisa Y Pang, BSc. PhD

Abstract: Despite significant advances in both our understanding and the treatment of cancer, the disease remains one of high mortality and morbidity in all species. Increase in survival times in human cancer have increased significantly in the past 25 years but most of these increases have been through small incremental changes. For some cancers, e.g. pancreatic cancer, survival times have not increased significantly in over 100 years. In veterinary oncology, we have seen major shifts in the management of cancer in companion animals. Increased availability of specialist centres, coupled with changing attitudes in owners and veterinarians, have meant that we have seen an improvements in veterinary cancer care borne from market pressures and increased awareness and understanding. In this review piece we will look at the changing face of cancer biology over the past 25 years, and consider the barriers to clinical progress in veterinary medicine. Finally, we will share an optimistic view of the future and the prospect for greater control over this devastating disease.

Veterinary Oncology: Biology, Big Data and Precision Medicine

Lisa Y. Pang, and David J. Argyle[1]

The Royal (Dick) School of Veterinary Studies and Roslin Institute, Easter Bush, Roslin, Midlothian, EH259RG, UK

Abstract

Despite significant advances in both our understanding and the treatment of cancer, the disease remains one of high mortality and morbidity in all species. Increase in survival times in human cancer have increased significantly in the past 25 years but most of these increases have been through small incremental changes. For some cancers, e.g. pancreatic cancer, survival times have not increased significantly in over 100 years. In veterinary oncology, we have seen major shifts in the management of cancer in companion animals. Increased availability of specialist centres, coupled with changing attitudes in owners and veterinarians, have meant that we have seen an improvements in veterinary cancer care borne from market pressures and increased awareness and understanding. In this review piece we will look at the changing face of cancer biology over the past 25 years, and consider the barriers to clinical progress in veterinary medicine. Finally, we will share an optimistic view of the future and the prospect for greater control over this devastating disease.

[1] Joint-winner of the Kennel Club Charitable Trust's International Award 2015

Introduction

According to data from Cancer research UK (CRUK), in 2012 there were 14.1 million new human cancer diagnoses world-wide and 8.2 million deaths[2]. Reducing cancer mortality is clearly an international priority. However, despite incremental improvements in cancer therapies, the disease remains one of high morbidity and mortality in all species (Argyle and Blacking, 2008). Improvements in public health and the control of infectious disease have compounded the problem making cancer the world's leading cause of death in humans. In addition, cancer has a huge impact on the economy through loss of productivity, loss of years of life, and cost related to treatment. According to American Cancer Society the total economic impact of premature death and disability from cancer worldwide was $895 billion in 2008[3]. This figure represents 1.5% of world's GDP and does not include direct cost of treating cancer. According to Murphy and Topel (2003), a 10% reduction in cancer deaths worldwide would be worth $4.7 trillion in social value.

Cancer in veterinary species can have two broad consequences. Cancer in livestock species can have a major economical impact, especially an infectious cause of cancer, e.g. Marek's disease in poultry, or Bovine Leukosis in cattle, causing significant loss of production. In contrast, the major impact on companion animals relates to their long-term health and their relationship with their owners. Although true epidemiological data worldwide is lacking in

[2] http://www.cancerresearchuk.org/health-professional/worldwide-cancer-statistics
[3] http://www.cancer.org

veterinary medicine, we estimate that the incidence of cancer in dogs is around 1 in 3 (and 1 in 4 to 5 in cats) (Pang, et al., 2009). This is not dissimilar to man and with a similar pattern of improved control of infectious disease pushing cancer up the league table of significant causes of death. Cancer treatments and (and consequently cancer treatment centres) have increased significantly in the last 20 years. Cancer treatments have become "accepted clinical practice" and owners now have much broader access to facilities such as external beam radiation. The control of cancer and cancer treatment-related side effects is much improved with the development of new drugs (e.g. NK-1 inhibitors for nausea) and we have seen the first targeted drugs for veterinary oncology being approved and launched (e.g. London et al., 2009). We have learnt a great deal about the biology of cancer in dogs and cats in the last two decades. This has been supported by the publications of species genomes which has also created, in small part, the tool box required to understand this disease at the genetic level and also investigate the clear breed predispositions for certain types of cancer (Ostrander and Kuglyak, 2000). However, as with human medicine, we still recognize cancer as the leading chronic disease and one of the biggest causes of death in companion animals (Argyle and Blacking, 2008).

The hallmarks of cancer

It is very difficult to define what a cancer is and to put that definition into a clinical context. If one considers that homeostasis is fundamental to health, then cancer can be considered in terms of a breakdown in the homeostatic mechanisms that control cell growth, cell division and cell death. Consequently,

we have to deal clinically with a group of cells, who have lost control of intrinsic cell growth and division, and can, under certain circumstances, spread (metastasize) to distant sites in the body. It is often this last critical step that can ultimately lead to the death of the patient.

Our traditional understanding of how a cancer develops comes from studies and mathematical modeling in diseases such as colon cancer in man (e.g. Little and Wright, 2003) and is built upon seminal work by Nordling (1953) and Knudson (1971). Colon cancer is one of the diseases that has allowed clinicians and scientists to model multistage carcinogenesis, demonstrating the changes from polyp formation to metastatic colon cancer. This model has been central to identifying key changes in cells that give rise to the malignant phenotype, from an initiation step (first fundamental genetic change to the DNA of the cell), and including the multiple stochastic genetic "hits" that the cell acquires to become a cancer cell (Figure 1). What is clear is that cancer is a disease that affects the fundamental genetic material (DNA) of a cell, the phenotype of which is passed to the daughter cell. The discovery of viruses that cause cancer laid the foundation for the discovery and description of oncogenes and tumour suppressor genes (Argyle and Blacking, 2008). These genes and their protein products are intimately involved with cell cycle regulation. Oncogenes are the cell's "accelerator pedal" and drive cell growth and division. Tumour suppressors are the cells "brake pedal" and add a level of control to the cell cycle. Cancers often contain major changes in these genes, which cause a breakdown in homeostasis, making them significant targets for therapy.

The almost exponential advances in molecular biology over the past 25 years have facilitated the dissection of these pathways and the development of drugs to target them. For a disease for which clinical control has been centred on the crudest of treatments (cancer chemotherapy), the advent of these discoveries sparked a fiercely competitive search for drugs that could target specific pathways that are known to be dysregulated in cancer.

However, what has become apparent, are the myriad of "altered" pathways and genetic changes in cancer cells that present a picture of a far more complex syndrome at the cellular level. In 2000 and again in 2011, Hannah and Weinberg made a significant attempt to distil the cancer phenotype into the acquisition of fundamental characteristics. The initial six cancer traits defined in the 2000 paper were added to in 2011, when the authors expanded the model to include evasion of the immune system and the acquisition of abnormal metabolic pathways (Figure 2). These traits are common across cancer phenotypes and offer the possibility of defining opportunities for biomarker discovery or therapeutic intervention. However, as we have developed the tools to define these pathways in detail, explore multiple genes in multiple cell types, define genetic and protein profiles, the complexity of the cancer cell seems to expand. As an added complication, both the cancer niche (microenvironment) and the epigenome have come to the foreground as being major players in cancer initiation and progression.

Challenging the traditional model of cancer development

In the last 10 years we have seen significant challenge to the traditional stochastic model of cancer development (described above). In many ways the simple model from initiation to metastatic cell (requiring the acquisition of multiple hits over time), did not fit well with our understanding of tissue and cell turnover in organ systems. An evolving model (cancer stem cell model) treats the cancer as an "organ system" where the bulk tumour population is driven by a small number of cancer stem cells (Blacking et al., 2007). This model has not been universally accepted (and may be different for different cancer types) but has gained significant ground in recent years. The clinical significance of this is immense as it gives the fundamental basis for tumour heterogeneity and suggests that a cancer is driven by cells that have striking resistance to conventional anti-cancer drugs. Cancer stem cells have been identified in cat and dog cancers that have significant resistance to conventional cancer drugs, radiation and have altered responses to DNA damage (Wilson et al., 2008; Pang et al., 2011, 2013 and 2015). The true classification of these cells is still controversial and there is still no universal cell marker for purification of these cells (Blacking et al., 2012). However, what is clear, is that cancers contain sub-populations of cells that are highly resistant to conventional therapies and contribute significantly to tumour heterogeneity and treatment failure (Figure 3).

Genes, dreams and cancer signatures

From a position over 20 years ago, when we could only look at single pathways or genetic changes in cancer cells in a stepwise fashion, we have moved to a position when we can examine thousands of genes in a cancer sample using gene

array “chips”. Initially, these were expensive technologies but the cost has plummeted in recent years, accompanied by newer technologies such as high throughput sequencing and RNA sequencing (RNA-seq). RNA-seq uses Next Generation Sequencing (NGS) to rapidly analyze the changing transcriptome in a cancer cell. This has been coupled with cost-effective and rapid ways of examining the cancer protein profile, its secretome, the metabolome and many of the epigenetic mechanisms operating at the cellular level. These technologies in cancer discovery have been used to:

1. Identify common cancer signatures across phenotypes
2. Identify potential targets for drug development
3. Identify “driver” and “passenger” mutations to assist drug discovery
4. Identify biomarkers of cancer for early detection
5. Identify specific pathways that may be druggable.

These technologies have also become affordable enough to be used to study companion animal tumours, both in their own right and as models for human disease (e.g. Mudaliar, et al., 2013; Pang et al., 2014). There is little doubt that the information obtained from these studies is proving incredibly useful. However, the challenge is still to be able to translate discovery into practical solutions for patients.

Why no cure?

We have experienced an exponential growth in understanding of cancer biology in the past 25 years. However, although we have seen some shift in survival times and improved mortality in humans, we have not seen the paradigm shift that the new cancer technologies promised. Pragmatically, this should not be a

surprise considering the complexity of the disease, but it is worth considering a number of issues that have arisen and how these may be overcome:

Data, data and more data: Our ability to dissect the cancer genome, proteome and metabolome has become incredibly refined and affordable. However, our ability to analyze the sheer volume of data (bioinformatics) has not kept pace with our ability to derive it. Much effort is now underway to expand our bioinformatics capability to keep pace with the information being gathered and to be able to use that information in a clinically relevant way. It is absolutely essential that cancer researchers and oncologists do not work in isolation but work across disciplines with bioinformaticians, mathematicians, engineers, and computer scientists, so we can both effectively mine and put some context to the enormity of the biological and clinical data that can now be generated.

Human colorectal cancer in man exemplifies the challenges that we face as cancer researchers and oncologists. Although colorectal cancer (CRC) was among the first solid tumors to undergo molecular profiling, the clinical translation of this knowledge into effective therapies has been impeded by the startling level of complexity and heterogeneity revealed among these tumours. Despite approval of several new drugs in recent years, the success of these and other agents in development has been stifled by the complex nature of CRC. It has become clear that the only way forward requires a paradigm shift toward integrative analyses that encompass multiple classes of genomic aberrations and consensus classification of CRC based on genomic data to facilitate more effective management of this disease.

Darwinian evolution: What has become very clear is that any "omic signature" gained for a specific cancer or biological sample reflects a simple snapshot in time for that sample. Expression of genes and proteins can rapidly change in a rapidly evolving tumour system and can be a reflection of inherent changes in the cell or as a result of changes in the cancer microenvironment (e.g. Greaves and Maley, 2012). This is hugely challenging as we may be identifying drug targets that are only transitory in nature or are subject to intense selection pressures. In addition to selection, there is also increasing evidence of significant cell plasticity in tumours (adaptation) that may also change the potential of druggable targets (Faurobert et al., 2015). It is clear that heterogeneity within tumours contributes significantly to treatment failure, but this heterogeneity is itself very dynamic and difficult to document in real-time (Brooks et al., 2015).

One of the major reasons for treatment failure in human and veterinary patients is the development of drug resistance. Drug resistance developing during treatment with conventional chemotherapy drugs is well documented in human and veterinary medicine and has been a subject of significant research investment. The development of targeted drugs which "hit" a specific pathway or "driver mutation" has been seen as a major breakthrough in cancer drug development, exemplified by the plethora of small molecules that have been developed to target the cancer kinome. Tyrosine kinases have been a hotly researched area of drug development as changes (e.g. mutations) in kinase pathways represent major drivers of malignancy (Bavcar and Argyle, 2012).

Imatinib (Gleevec) is a small molecule inhibitor that targets Receptor Tyrosine Kinases (RTK) and was one of the fastest cancer drugs to reach the market (from initial discovery to clinical licensing), being used extensively in human leukaemia. However, as with conventional drugs, the selection pressure created by using one single drug supports the development of drug resistance in certain groups of patients (Bixby and Talpaz, 2011). The development of Imatinib has been followed by the development of second and third generation RTK inhibitors to overcome the inevitable acquisition of resistance. However, as we have described above, cancer is far more complex and just targeting one driver mutation in a tumour is probably insufficient. It is likely that the greatest success in cancer control is going to be achieved through targeting multiple pathways in cancer and also playing close attention to tumour microenvironment and the role of epigenetic drivers in cancer.

The concept of tumour evolution also applies to how the body's immune system responds to cancer and how successful immunotherapy is in cancer patients (Figure 4). As with targeted drug therapy, advances in immunotherapy have resulted in remarkable clinical responses in some human patients (Raposo, et al., 2015). However, one of the biggest challenges in cancer therapeutics is the development of resistant disease and disease progression on or after therapy. For patients with metastatic cancer, conventional chemotherapy (plus or minus targeted therapies) has not proven curative. However, there is significant clinical trial data in human patients to suggest that immunotherapy has the potential to achieve long lasting remissions in patients with metastatic disease. However, as with some of the targeted therapies, immune-selective pressure for

resistant tumour cells clearly exists (Restifo et al., 2016). It is likely that this resistance derives from the type of Darwinian evolution described above (e.g. selection pressure on the tumour giving rise to selective loss of components of MHC). In addition, tumour cells may acquire resistance through adaptation in response to interactions with immune cells. One mechanism that has gained prominence recently has been the tumour cell expression of programmed cell death protein (PD1) and its ligand (PDL1), which serve to down regulate the anti-tumour immune response (Mamalis, et al., 2014). Drugs and monoclonal antibodies targeting this "immune checkpoint" are the subject of intense research and human clinical trials.

"Big bang theory" and tumour heterogeneity: Recent studies of colon cancer utilizing genomic data and mathematical modeling, suggest that the majority of genetics changes and intratumoural heterogeneity (ITH) actually occurs very early on in tumour evolution once the malignant phenotype of the cell has been achieved (Sottoriva et al., 2016). This also suggests that a tumour's ability to invade and metastasize are programmed early in development rather than acquired by selective forces. This has major implications for drug and biomarker discovery as it suggests that the formation of new driver mutations during tumour evolution are not as common as once considered. It also means that some tumours are just "born bad" whatever we do to them

The lack of good model systems: Rodent xenograft models have been the traditional test bed for new anti-cancer therapies. However treatment responses in rodents frequently do not translate into benefit in patients (Pang and Argyle,

2009). This mismatch is multifactorial but broadly reflects major differences in tumour biology and pathophysiology and lack of tools to measure critical changes in the tumour microenvironment that drive tumour growth and response to treatment. Basic cancer research, combined with xenograft models have made great progress in our understanding of the mechanisms that underlie the development of human cancer and in cancer detection but the current pre-clinical models are too slow, too costly and lack predictability for the efficient translation into new cancer treatments. Similarly, small animals are insufficient for the development of new technology for detecting early cancers. Mouse models have played an important role in identifying the molecular pathways of cancer but the uncertainty of artificial tumours in mice to foresee the clinical outcome of new treatments and their insufficiency for testing new imaging technology have become ever tighter bottlenecks for bringing new treatments and technology to the benefit of the patients. Hence, new pre-clinical models to more rapidly translate advances in basic cancer research, diagnostics and treatment into the clinic are of most urgent need.

A cause for optimism?

Our ability to dissect the cancer genome and all of its components has far exceeded our ability to analyze and understand the data. We can therefore conclude that the complexity of the cancer cell is currently impeding our ability to define and produce better treatments and better outcomes for patients. As a community involved in cancer research, clinical oncology or both, what can we do to drive progress and is there cause for optimism? The simple answer to this is that there is great deal we can do and there is definitely cause for optimism in

both human and veterinary oncology. We are seeing a renaissance and rejuvenated interest in conventional treatments such as radiotherapy, we are developing new and innovative ways to study cancer, and more than ever before we are exploring cancer without any species boundaries. Below is not an exhaustive list, but offers an optimistic view of veterinary and human oncology:

Advances in conventional therapies: Patient responses to conventional treatments in veterinary oncology have become more predictable as we gain greater experience in managing common cancer types. However, for diseases such as Lymphoma, we have probably reached a "watershed" in terms of our ability to significantly alter disease free interval and survival times with the drugs we have available (Comazzi, et al., 2015). This is also considering our appropriate need in veterinary oncology to maintain quality of life in our patients. New cancer chemotherapy dugs are few and far between and we rely on orphan drugs from human medicine to fill the significant pharmacy gap that we have in veterinary oncology. We have, however, seen a major renaissance in radiation oncology, especially in terms of availability. We have gone beyond course fractionated regimes and embraced radiotherapy plans and prescriptions with curative intent. This is only set to increase with advances in planning systems and increased use an availability of IMRT (Intensity Modulated Radiotherapy) and SBRT (Stereotactic Body Radiotherapy) (Feng, et al., 2015 and 2016)

Advances in imaging: In recent years there has been a tremendous improvement in imaging technologies and access to these technologies. We have

been able to go beyond radiographic analysis and been able to take advantage of the imaging resolutions afforded by Computerized Axial Tomography (CT) and Magnetic Resonance Imaging (MRI). While these modalities are improving the imaging resolution in terms of anatomy, functional imaging (e.g. Positron Emission Tomography (PET)) is set to become more available and will be a major diagnostic modality, especially for cancer patients and for the identification of primary and metastatic lesions. The cost and availability of new modalities is coming down and we can expect that these will become a common part of the cancer staging process both in primary care and referral centres.

Drug and device development: New drug development for cancer in companion animals is hugely challenging, not least for even the biggest pharmaceutical companies. Since the launch of toceranib (Palladia) and masitinib (Masivet), there have been no new "second generation" drugs as seen in human oncology. The indications for both of these drugs (as dictated by the license arrangement) was somewhat limited and was not the panacea for cancer that some may have wanted or predicted. We are still (as a community) learning a lot about how to use these drugs either alone or in combination with conventional drugs, and it is possible that their use will become more widespread in these scenarios. Dogs do develop resistance and with few follow-on options (no second generation drugs), their use can become limited in some patients. However, for the veterinary pharmaceutical industry the financial margins on these drugs and the expense of getting them to market are a huge challenge, especially when you consider the size of the market. The veterinary oncology market is a mere fraction of the $100 billion dollar human cancer drug

market. A secondary route to market could involve using drugs developed for human oncology, as long as pharma can tolerate the potential price differential between what they can charge for a human drug and what can be reasonably charged for a veterinary drug.

However, instead of human and veterinary oncology drug development operating in parallel, there is a model that transcends the species boundaries to allow combined drug development. Rodent xenograft models have been the traditional test bed for new anti-cancer therapies. However treatment responses in rodents frequently do not translate into benefit in patients. This mismatch is multifactorial but broadly reflects major differences in tumour biology and pathophysiology and lack of tools to measure critical changes in the tumour microenvironment that drive tumour growth and response to treatment. Basic cancer research, combined with xenograft models have made great progress in our understanding of the mechanisms that underlie the development of human cancer and in cancer detection but the current pre-clinical models are too slow, too costly and lack predictability for the efficient translation into new cancer treatments (Pang and Argyle, 2009). Similarly, small animals are insufficient for the development of new technology for detecting early cancers. Mice models have played an important role in identifying the molecular pathways of cancer but the uncertainty of artificial tumours in mice to foresee the clinical outcome of new treatments and their insufficiency for testing new imaging technology have become ever tighter bottlenecks for bringing new treatments and technology to the benefit of the patients. Hence, new pre-clinical models to more rapidly translate advances in basic cancer research, diagnostics and treatment into the

clinic are of most urgent need. Spontaneous or naturally occurring tumours in dogs and cats share important molecular, histopathological and therapeutic characteristics with corresponding human disease and, thus, provide cancer models that are closer to man than rodent models (Rowell et al., 2011; Shearin and Ostrander 2010; Khanna et al., 2006; Pang and Argyle, 2009). Clinical data derived from trials in spontaneous tumours in domestic animals could serve not only to improve animal health but serve as an important link between basic cancer research and human and veterinary clinical trials. While much emphasis has been placed recently on translation of biology into clinical practice, this kind of approach aims to create a platform of inderdisciplinarity that supports both translation, and transformation of clinical cancer practice, offering the greatest opportunity for Impact. This would include:

1. Reducing the time taken for a therapeutic targets to be translated into clinical benefit
2. Reducing the high costs of therapeutic development
3. Increasing the predictability of human pre-clinical models.

This concept can go beyond drug development and also be applied to other aspects of cancer research such as the development of medical devices. As an example, IMPACT (Implantable Microsystems for Personalized Anti-cancer Therapy)[4] is a collaboration between engineering, veterinary oncology, human oncology, chemistry, and social science, to develop implantable sensors that are able to detect changes in tumour microenvironment in real time. For example, if we can detect subtle changes in hypoxia in real-time during radiotherapy, then

[4] http://www.impact.eng.ed.ac.uk

treatment plans can be adjusted rapidly to compensate and improve clinical outcomes in patients. This project aims to develop a platform technology that could be applied to a wide range of cancers and perhaps ultimately being able to deliver anti-cancer drugs locally, and in a controlled way.

Monoclonal antibodies for diagnosis and treatment: The development of small molecules to target RTK pathways and driver mutations was considered to be one of the major breakthroughs in cancer research. However, monoclonal antibodies have now far exceeded small molecules in terms of the market share of biologics being used in cancer treatments. Some of the advantages of monoclonal antibody therapeutics over conventional drugs are high specificity, precise mode of action and long half-life, which favours infrequent dosing of the antibody. Monoclonal antibodies have been developed for a number of cancer targets including Anti-CD20 (B cell Lymphoma, Anti-EGFR (multiple targets including head and neck cancer) and anti-VEGFR (Multiple cancer types targeting angiogenesis) (reviewed by Xin et al., 2013). However, the use of "human" monoclonal antibodies in veterinary oncology is usually not feasible due to the development of an immune response to foreign protein. Recently new techniques have allowed the development of species-specific (e.g. caninized) monoclonal antibodies. A full description of this technology is outwith the scope of this review but can be found by Breiro et al., 2016). Caninized anti-CD20 is in clinical use and a pipeline of discovery through to clinical application is being developed by a number of companies in the veterinary arena (Jain et al., 2016). This is a truly exciting prospect, as it will deliver new and affordable reagents to the veterinary oncology community.

A renaissance for immunotherapy: Immunotherapy for cancer in all species has followed a continuous sine wave varying between optimism and pessimism. Immunotherapy has become one of oldest forms of cancer treatment, the aim being to harness the body's immune system to target a tumour with altered "self proteins". While immunotherapy has achieved considerable success in some patients, we still do not fully understand why some patients will mount a positive anti-tumour response, and others do not. This is also confounded by Darwinian selection pressures (described above) and the development of adaptive responses to immunotherapy. As with our understanding of the molecular events in cancer, our understanding of immunity is also exponentially increasing. There is particular cause for optimism currently around the dissection of the pathways involved in adaptive responses and a good example of this is the PD1/PD1L axis. Programmed death-1 (PD-1) is expressed on the surface of immune cells, and programmed death ligand-1 (PD-L1) is often expressed on cancer cells. When PD-1 and PD-L1 bind, this results in suppression of T cell activity and reduction of T cell-mediated cytotoxicity (Robert et al. 2014). Thus, PD-1 and PD-L1 are immune down-regulators or immune checkpoint "off switches" (Mamalis et al., 2014), which allow cancer cells to evade immune destruction. Anti-PD1 and PD1L drugs and monoclonal antibody development have been intensely pursued by the pharmaceutical and academic communities as a mechanism for immune-modulating cancer patients (e.g. in malignant melanoma). Whereas previous immunotherapies have focused on promoting anti-tumour immunity, this approach tries to inhibit immune checkpoints that protect cancers from immune destruction. Alone, this therapy may be insufficient to offer complete cures, but combining it with other

441 modalities or immunotherapies may offer a significant advantage over current
442 treatments.

443 **Big data and precision medicine:** The development of the appropriate
444 reagents for mining veterinary genomes, proteomes and metabolomes is rapidly
445 expanding. Coupled with this is the reduction in costs associated with
446 sophisticated genomic and proteomic analysis. With this will come an increased
447 ability to:

448 1. Mine veterinary cancer genomes and proteomes using multiple samples.
449 2. Potentially identify biomarkers for the early detection of cancer, prediction of
450 treatment success or the early detection of treatment failure.

451 These technologies are already is use and proving useful for dissecting the
452 complexity of cancer. However, with this we must embrace the importance of
453 bioinformatics, statistics and mathematical modeling if we are going to take full
454 advantage of the amount of data we are generating. This must also be linked
455 with appropriate clinical data from the field so we can develop appropriate
456 algorithms that will be useful clinically. This will require a paradigm shift in how
457 we traditionally approach veterinary medicine:

458 1. We must improve how we record and collect clinical data. We suffer in
459 veterinary medicine with low patient numbers compared to human medicine
460 and this is challenging when we need large cohorts of patients for specific
461 studies. With this, there will be a requirement for national and international
462 collaboration, standardization of clinical recording, and significant
463 investment in biobanking resources. Some of these are being addressed in
464 some part, but this will require significant funding and organization. The

concept of "Big Data" is being embraced by human medicine and, as a profession, if we are going to retain a competitive edge we must also embrace this.

2. We must break down the discipline barriers and develop systems to handle large data sets. This will involve developing systems that will allow us to integrate clinical, biological and epidemiological data to provide the optimum clinical care for our patients (precision medicine). This may involve mapping a specific "comparative oncology ecosystem" that will provide the framework for interdisciplinarity and collaborative research.
3. In embracing new technologies, we must also consider how we train the next generation of veterinarians to ensure they know how to interpret the potentially large amounts of data they will be able to generate from an individual patient.
4. In the earlier years of the twentieth century, we relied up symptom recognition and application of knowledge. Today, we are more in tune with pattern recognition and application of the evidence base. Tomorrow, it is likely that we will embrace the acquisition of multiple levels of patient data (genome, to phenome) and apply that knowledge and information to treatment, but based up on specific algorithms derived from an evidence base. This will herald the dawn of precision veterinary medicine (Figure 5).

There is much cause for optimism in this arena as we are in the early stages of developing some of these systems to achieve this end goal. Our challenge will be to work collaboratively and to ensure these approaches are adequately funded.

Concluding remarks

At the start of this synopsis, I painted a rather challenging view of cancer research and clinical oncology where complexity of this disease will constantly hinder progress. However, I strongly believe that many of the hurdles that I have described can be overcome to the benefit of all species. As a community, we must think far beyond the translation of basic biology into clinical practice, and consider the defining research and application that will truly transform clinical practice to the benefit of patients. We have to remove the boundaries to research silos that are restricting progress and also the traditional species boundaries between human and veterinary oncology. As an example, data science and large data set analysis will be vital to understanding the complexity of cancer at the cell and population level. We will need to integrate clinical and biological data to improve treatment outcomes and design specific therapies. Precision medicine has been coined in human medicine as a model that proposes the customization of healthcare, with medical decisions, practices, and/or products being tailored to the individual patient. It is possible, with new technologies that veterinary medicine will have to move in a similar direction. However, we have to embrace new technology and work collaboratively across disciplines to achieve this.

Acknowledgements

The authors' work is currently and has been generously funded by the Petplan Charitable Trust, The RCVS Trust (now RCVS Knowledge), The Wellcome Trust, The BBSRC, the EPSRC and Dogs Trust. David Argyle is in grateful receipt of an International Canine Health Award (made possible by a grant from Vernon and Shirley Hill) from the Kennel Club Charitable Trust to extend his research on

canine cancer.

Conflict of Interest

The Authors have no conflict of interest

References

Argyle D.J. and Blacking T.M. 2008. From Viruses to Cancer Stem Cells: Dissecting the Pathways to Malignancy. The Veterinary Journal. 177, 311-23.

Bavcar S, Argyle DJ. 2012. Receptor tyrosine kinase inhibitors: molecularly targeted drugs for veterinary cancer therapy. Veterinary and Comparative Oncology 10, 163-73.

Bixby D, and Talpaz M. 2011. Seeking the causes and solutions to imatinib-resistance in chronic myeloid leukemia. Leukemia 25, 7–22

Blacking T.M., Wilson, H. and Argyle D.J. 2007. Is Cancer a Stem Cell Disease. Theory, Evidence and Implications. Veterinary and Comparative Oncology 5, 76-89.

Blacking, T.M., Waterfall, M. and Argyle D.J. 2012. Flow cytometric techniques for detection of candidate cancer stem cell subpopulations in canine tumour models. Veterinary and Comparative Oncology 10, 252-73.

Brooks M.D., Burness, M.L., Wicha, M.S. 2015. Therapeutic Implications of Cellular Heterogeneity and Plasticity in Breast Cancer. Cell Stem Cell, 17, 260–271,

Faurobert E, Bouin AP, Albiges-Rizo C. 2015. Microenvironment, tumor cell plasticity, and cancer. Current Opinion in Oncology 27, 64-70.

Feng, Y., Welsh, D., McDonald, K., Carruthers, L., Cheng, K., Montgomery, D., Lawrence, J., Argyle, D.J., McLaughlin, S., McLaren, D.B., et al. 2015. Identifying the dominant prostate cancer focal lesion using image analysis and planning of a simultaneous integrated stereotactic boost. Acta Oncologica 54, 1543-50

Feng, Y., Lawrence, J., Cheng, K., Montgomery, D., Forrest, L., Mclaren, D.B., Mclaughlin, S., Argyle, D.J., Nailon, W.H. 2016. Image Registration In Veterinary Radiation Oncology: Indications, Implications, And Future Advances. Veterinary Radiology and Ultrasound. Jan 18. doi: 10.1111/vru.12342. [Epub ahead of print]

Greaves, M., Maley, C.C. 2012. Clonal Evolution In Cancer. Nature. 481, 306-313.

Hanahan, D., Weinberg, R.A. 2000. The hallmarks of cancer. Cell 100, 57–70.

Hanahan, D., Weinberg, R.A. 2011. Hallmarks of cancer: the next generation. Cell 144, 646–74.

Jain, S., Aresu, L., Comazzi, S., Shi, J., Worrall, E., Clayton, J., Humphries, W., Hemmington, S., Davis, P., Murray, E., et al. (2016) The Development of a Recombinant scFv Monoclonal Antibody Targeting Canine CD20 for Use in Comparative Medicine. PLoS One. Feb 19; 11, e0148366.

Khanna, C; Lindblad-Toh, K; Vail, DM; et al. (2006) The dog as a cancer model. Nature Biotechnology: 24: 9: 1065-1066

571

572 Knudson, A. 1971. Mutation and cancer: statistical study of retinoblastoma.
573 Proceedings of the National Academy of Sciences U S A 68, 820–823.
574

575 Nordling, C. 1953. A new theory on cancer-inducing mechanism. British Journal
576 of Cancer 7, 68–72.
577

578 Little, M.P. Wright, E.G. 2003. A stochastic carcinogenesis model incorporating
579 genomic instability fitted to colon cancer data. Mathematical Biosciences. 183,
580 111–134
581

582 London, C.A., Malpas, P.B., Wood-Follis, S.L., Boucher, J.F., Rusk, A.W., Rosenberg,
583 M.P., Henry, C.J., Mitchener, K.L., Klein, M.K., Hintermeister, J.G., et al. 2009.
584 Multi-center, placebo-controlled, double-blind, randomized study of oral
585 toceranib phosphate (SU11654), a receptor tyrosine kinase inhibitor, for the
586 treatment of dogs with recurrent (either local or distant) mast cell tumor
587 following surgical excision. Clinical Cancer Research 15, 3856-65.
588

589 Mamalis, A., Garcha, M., Jagdeo, J. 2014. Targeting the PD-1 pathway: a promising
590 future for the treatment of melanoma. Archives of Dermatology Research 306,
591 511-9.
592

593 Mudaliar MA, Haggart RD, Miele G, Sellar G, Tan KA, Goodlad JR, Milne E, Vail DM,
594 Kurzman I, Crowther D, Argyle DJ. 2013. Comparative gene expression profiling
595 identifies common molecular signatures of NF-κB activation in canine and

596 human diffuse large B cell lymphoma (DLBCL). PLoS One. 8(9):e72591
597
598 Murphy, K., Topel, R. 2000. Diminishing returns? The costs and benefits of
599 improving health. Perspectives Biology and Medicine 46, S108-28
600
601 Ostrander, E.A. and Kruglyak, L. 2000. Unleashing the Canine Genome. Genome
602 Research 10: 1271-1274.
603
604 Pang, L.Y., Argyle, D.J. 2009. Using naturally occurring tumours in dogs and cats
605 to study telomerase and cancer stem cell biology. Biochimica et Biophysica Acta
606 1792, 380-91.
607
608 Pang, L.Y., Cervantes-Arias, A., Else, R.W., Argyle, D.J. 2011. Canine Mammary
609 Cancer Stem Cells are Radio- and Chemo- Resistant and Exhibit an Epithelial-
610 Mesenchymal Transition Phenotype. Cancers 3, 1744-62
611
612 Pang, L.Y., Blacking, T.M., Else, R.W., Sherman, A., Sang, H.M., Whitelaw, B.A.,
613 Hupp, T.R., Argyle, D.J. 2013. Feline mammary carcinoma stem cells are
614 tumorigenic, radioresistant, chemoresistant and defective in activation of the
615 ATM/p53 DNA damage pathway. The Veterinary Journal. 196, 414-23.
616
617 Pang, L.Y., Gatenby, E.L., Kamida, A., Whitelaw, B.A., Hupp, T.R., Argyle, D.J. 2014
618 Global gene expression analysis of canine osteosarcoma stem cells reveals a
619 novel role for COX-2 in tumour initiation. PLoS One 8; e83144.

620

621 Pang, L.Y., Argyle, D.J. 2015. The evolving cancer stem cell paradigm:

622 Implications in veterinary oncology. The Veterinary Journal. 205, 154-60

623

624 Raposo, T.P., Beirão, B.C., Pang, L.Y., Queiroga, F.L., Argyle, D.J. 2015.

625 Inflammation and cancer: till death tears them apart. The Veterinary Journal.

626 205, 161-74.

627

628 Restifo, N.P., Smyth, M.J., Snyder, A. 2016. Acquired resistance to

629 immunotherapy and future challenges. Nature Reviews Cancer, 16, 121-6.

630

631 Robert, C., Ribas, A., Wolchok, J.D., Hodi, F.S., Hamid, O., Kefford, R., Weber, J.S.,

632 Joshua, A.M., Hwu, W.J., Gangadhar, T.C. et al. 2014. Anti-programmed-death-

633 receptor-1 treatment with pembrolizumab in ipilimumab-refractory advanced

634 melanoma: a randomised dose-comparison cohort of a phase 1 trial. Lancet 384,

635 1109-17.

636

637 Rowell, J.L., McCarthy, D.O., Alvarez, C.S. Et al. 2011. Dog Models of naturally

638 occurring cancer. Trends In Molecular Medicine 17, 380-388

639

640 Shearin, A.L.; Ostrander, E. A. 2010 Leading the way: canine models of genomics

641 and disease. Disease Models & Mechanisms 1-2, 27-34

642

643 Sottoriva, A., Kang, H., Ma, Z., Graham, T.A., Salomon, M.P., Zhao, J., Marjoram, P.,

644 Siegmund, K., Press, M.F., Shibata, D., et al. 2015. A Big Bang model of human
645 colorectal tumor growth. Nature Genetics 47, 3209-16
646
647 Wilson, H.M., Huelsmeyer, M., Chun, R.A., Young, K.M., Friedrichs, K., Argyle, D.J.
648 2008. Isolation and characterization of Cancer Stem Cells from Canine
649 Osteosarcoma. The Veterinary Journal. 175, 69-75.
650
651 Xin, L., Cao, J., Cheng, H., Zeng, F., Hu, X., Shao, J. 2013. Human monoclonal
652 antibodies in cancer therapy: a review of recent developments Frontiers in
653 Bioscience 18:765-72.
654
655

Figure Legends:

Figure 1: The Stochastic and Traditional Model of Cancer Development: This supports that a cell within the body sustain an "initiation" event, which causes a damage and change to the cell's DNA (loss of gain of function of oncogenes or tumour suppressor genes). In most cells receiving such damage, the cell would either die by programmed cell death or arrest so that the cell could repair it's DNA. In cell's where this fails, they can accumulate genetic "hits" ultimately leading to the development of a cell with a malignant phenotype and the ability to metastasize.

Figure 2: The Hallmarks of cancer as proposed by Hannah and Weinberg (adapted). The model suggests that all cancers can be defined by the acquisition of 6 fundamental characteristics. In 2011, altered metabolism and evasion of the immune system were also included as enabling characteristics of cancer cells.

Figure 3: The stem cell model of cancer is not universally accepted and may be different for different cancer types. In the model proposed in this diagram, an adult stem cell is the target cell, which receives the initial genetic "hit" or "hits" which allows "reprogramming of the cell" to a primitive phenotype (Tumour Initiating Cell or TIC). This has been likened to the development if induced pluripotency in somatic cells in culture. Once established the tumour resembles an organ structure in that the bulk of the tumour (Daughter Cancer Cells, DCCs) is driven by a very small population of

cancer stem cells (CSC) that are capable of self-renewal. There is also emerging evidence that there is considerable plasticity in these cells that contribute to supporting metastatic spread.

Figure 4: The tumour is subjected to intense Darwinian selection pressures, both in terms of selection of phenotypes resistant to drugs or cell death, but also refractory to immune surveillance. Within this model, evolving tumour heterogeneity is compounded by cellular adaptation. This results in a very complex problem for the development of treatments for cancer.

Figure 5: The Development of Precision Veterinary Medicine. In the earlier years of the twentieth century we relied upon symptom recognition and application of intuition. Today, we are more in tune with pattern recognition and application of the evidence base. Tomorrow, it is likely that we will embrace the acquisition of multiple levels of patient data (genome, to phenome) and apply that knowledge and information to treatment, but based up on specific algorithms derived from an evidence base.

Figure 1

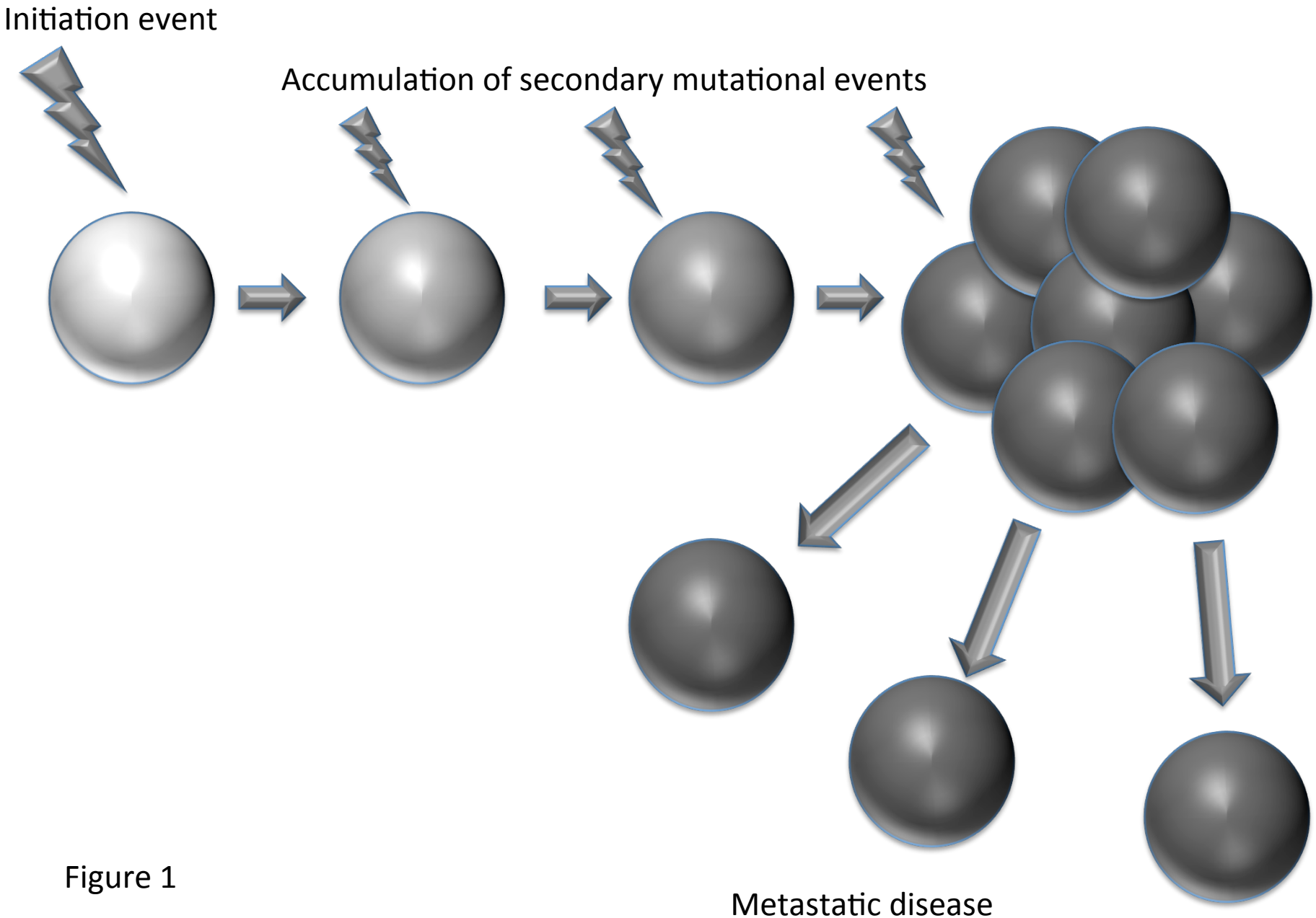

Figure 1

Figure 2

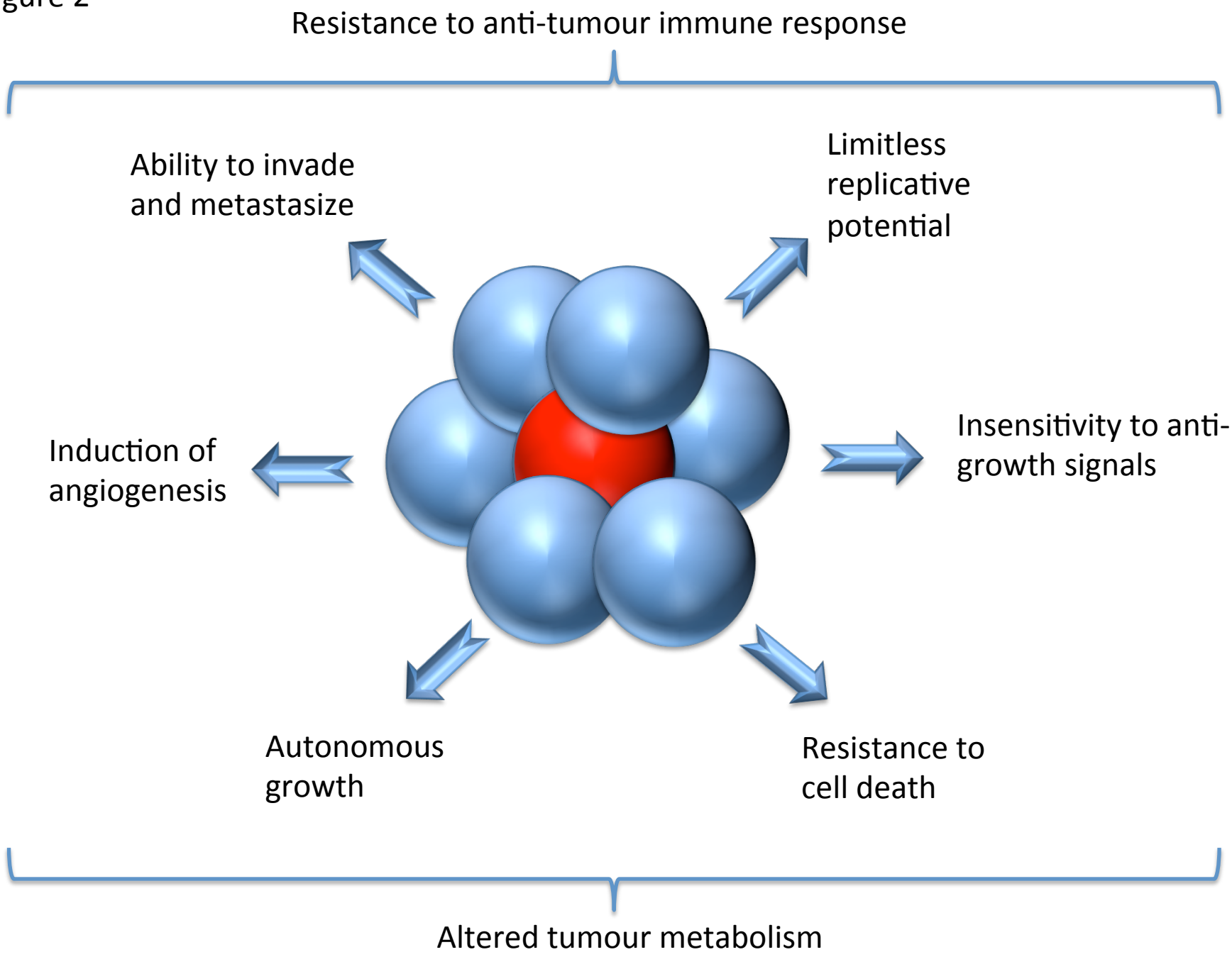
Resistance to anti-tumour immune response
Ability to invade and metastasize
Limitless replicative potential
Induction of angiogenesis
Insensitivity to anti-growth signals
Autonomous growth
Resistance to cell death
Altered tumour metabolism

Figure 3

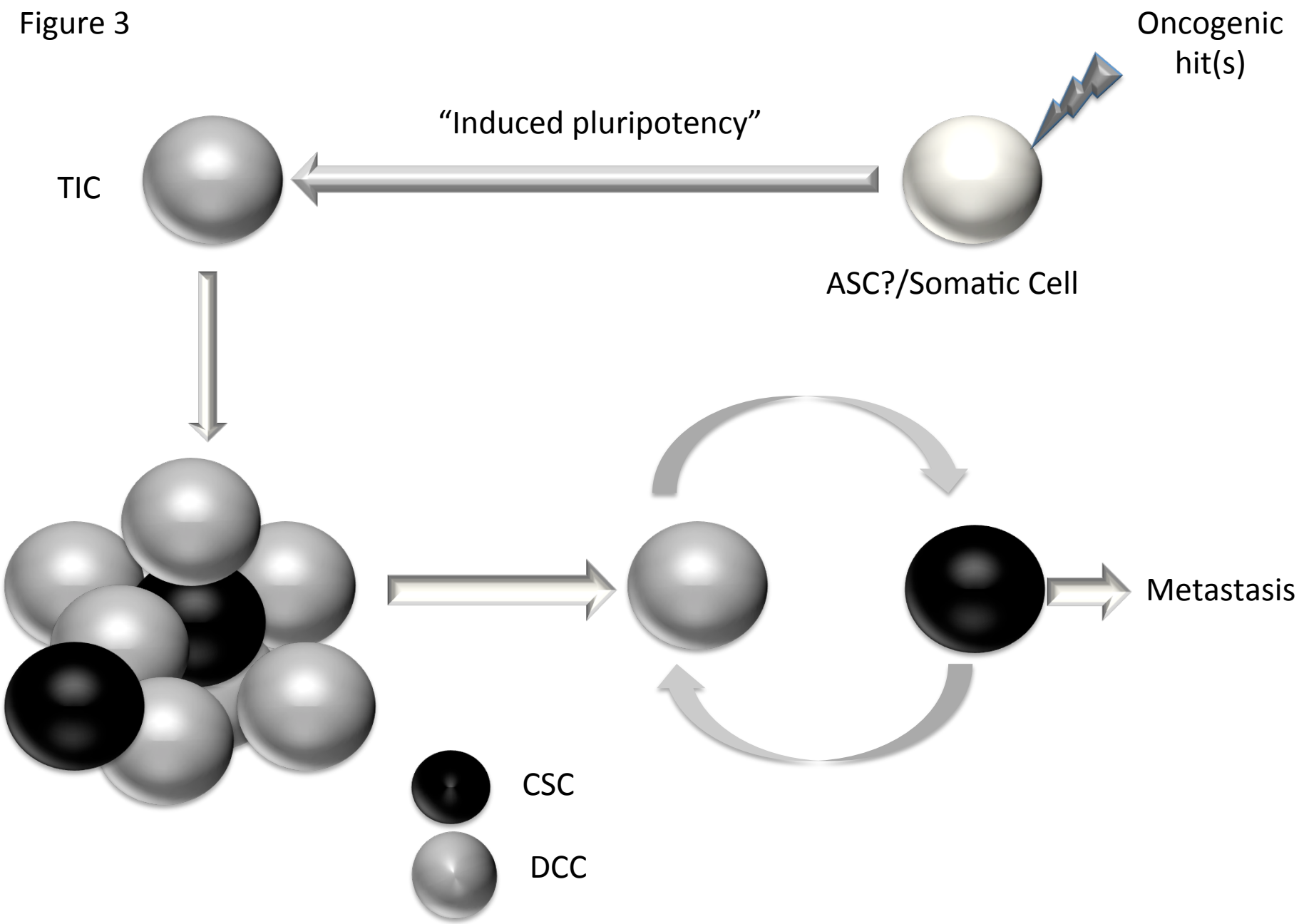
Figure 3
Oncogenic hit(s)
"Induced pluripotency"
TIC
ASC?/Somatic Cell
Metastasis
CSC
DCC

Figure 4

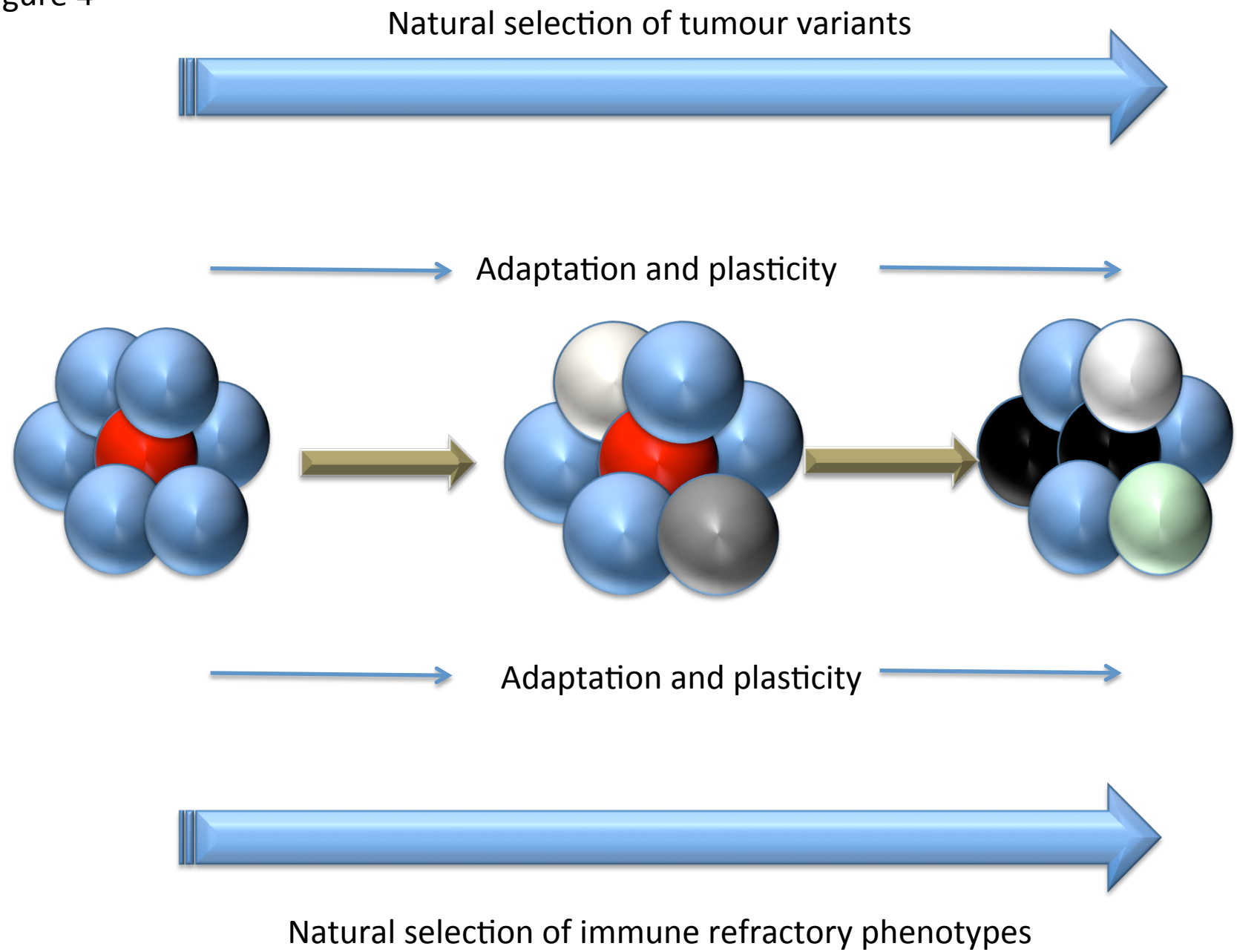
Natural selection of tumour variants
Adaptation and plasticity
Adaptation and plasticity
Natural selection of immune refractory phenotypes

Figure 5

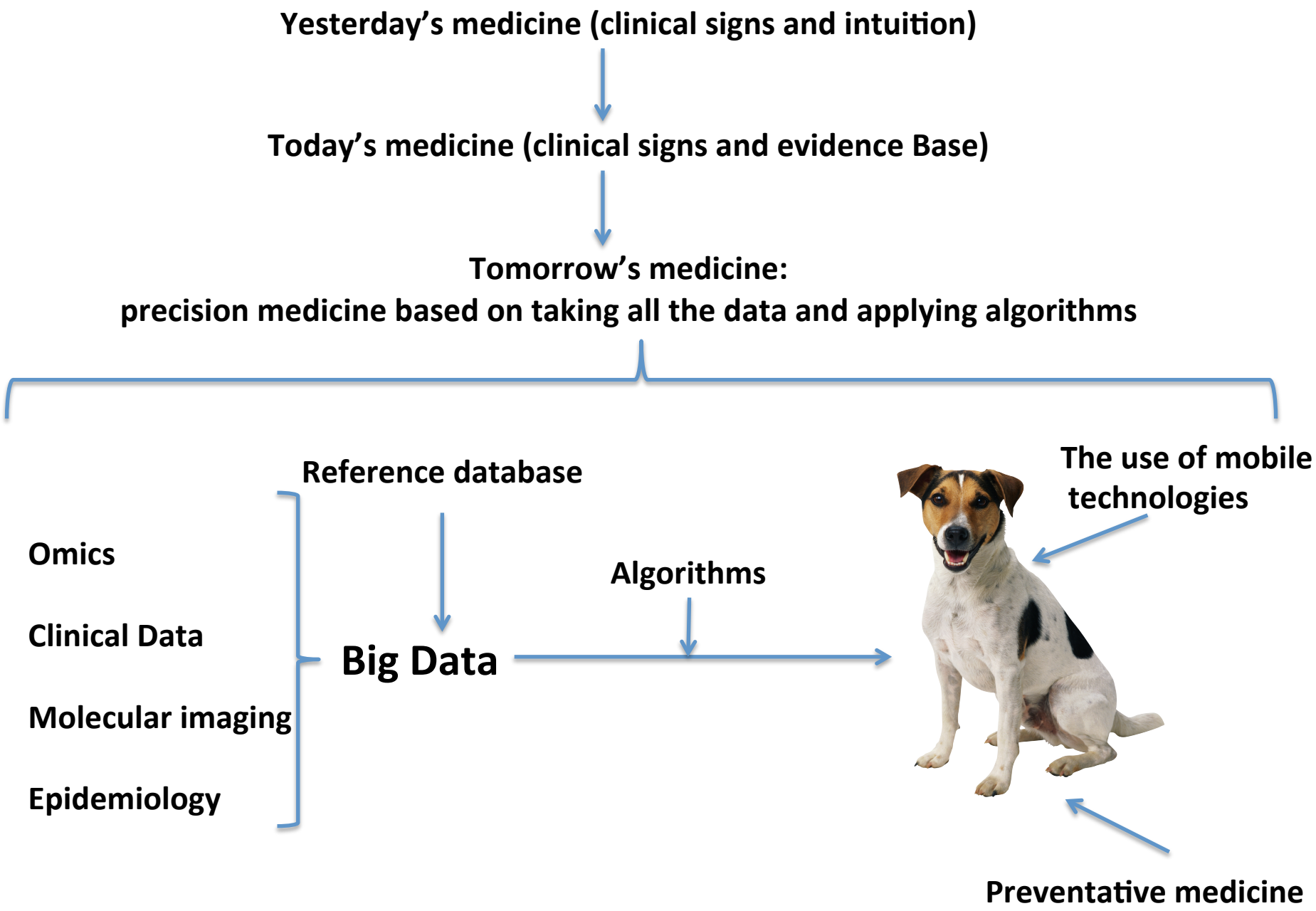

Figure 5

Highlights for Review

- Our understanding of cancer has increased exponentially in the past 25 years
- Our treatment of cancers in domestic animals has greatly improved
- Our ability to generate data about cancer exceeds our capacity to analyse it
- Much effort is needed to bring disciplines together to understand large data sets in cancer as they are too complex to be considered in isolation
- As we move forward in veterinary medicine, we will become more reliant on ways to quickly assimilate data from multiple sources in order to make appropriate clinical judgements.