The Veterinary Journal 205 (2015) 238–243

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Review

# Perspectives on the design of clinical trials for targeted therapies and immunotherapy in veterinary oncology

Laura Marconato [a,*], Paolo Buracco [b], Luca Aresu [c]

[a] Centro Oncologico Veterinario, via San Lorenzo 1-4, 40037 Sasso Marconi (BO), Italy
[b] Department of Veterinary Sciences, University of Turin, Largo Paolo Braccini 2-5, 10095 Grugliasco (TO), Italy
[c] Department of Comparative Biomedicine and Food Science, University of Padova, Viale dell'Università 16, 35020 Agripolis Legnaro (PD), Italy

ARTICLE INFO

*Article history:*
Accepted 25 February 2015

*Keywords:*
Targeted therapy
Immunotherapy
Clinical trials
Endpoints
Oncology
Veterinary

ABSTRACT

The field of oncology research has undergone major changes in recent years. Progress in molecular and cellular biology has led to a greater understanding of the cellular pathways and mechanisms of cell proliferation and tissue invasion associated with cancer. New classes of cancer therapies are becoming available or are in development but these new agents require a paradigm shift in the design of oncology clinical trials. This review provides an overview of clinical trial designs for the development of tumour vaccines and targeted therapeutic agents. In addition, some of the successes, limitations and challenges of these trials are discussed, with a special emphasis on the difficulties and particularities that are encountered in veterinary medicine compared to similar work in human patients.



## Introduction

Clinical trials in oncology represent a critical link between basic science research and clinical practice. Because standard chemotherapy has usually limited efficacy against most cancers, there has been an effort over recent decades to develop targeted agents and cancer vaccines that have led to significant improvement in outcomes for various cancers in humans (Zhang et al., 2009; Raval et al., 2014). Likewise, efforts have been pursued in veterinary oncology, and novel promising cancer therapeutics consisting of targeted agents (Hahn et al., 2008; London et al., 2009) and cancer vaccines (Bergman, 2010; Denies and Sanders, 2012) represent some of the most exciting opportunities in veterinary oncology.

Targeted therapy is used to describe agents that affect neoplastic cells and usually spare normal cells by interfering with specific molecules required for tumour development and growth (the so-called therapeutic targets). In humans, targeted agents include monoclonal antibodies (mAbs) and tyrosine kinase inhibitors (TKIs), and these are already major treatment options for cancer together with cytotoxic chemotherapeutic agents (Imai and Takaoka, 2006). The advantage of these drugs is the improved efficacy and selectivity they offer by blocking specific mechanisms involved in malignant transformation and progression. Validated therapeutic targets include membrane receptors playing a direct role in cancer biology, components of cytoplasmic signalling pathways, cell cycle regulator proteins, circulating growth factors, and proteins or factors involved in angiogenesis (Traxler, 2003).

Approved veterinary targeted drugs include toceranib and masitinib, which target, among others, the KIT receptor (Hahn et al., 2008; London et al., 2009). Both exhibit high response rates as single agents in canine mast cell tumours with the targeted *KIT* mutation (Bonkobara, 2015). Particularly, toceranib yielded higher response rates in dogs with mutated vs. wild-type c-*KIT* (69% and 37%, respectively) (London et al., 2009). Masitinib significantly prolonged survival in dogs with mutated vs. wild-type c-*KIT* (417 and 182 days, respectively) (Hahn et al., 2008).

In contrast to TKIs, mAbs generally have a higher specificity for their targets, a longer half-life (allowing for monthly administrations), and can be optimised using recombinant and protein technologies to improve their properties (Douthwaite and Jermutus, 2006). Several mAbs are approved for human use and an increasing number are currently in development, further highlighting the success of this approach. To reduce the risk of adverse immune responses, humanised mAbs and even fully human antibodies are currently the preferred approach. Antibody–drug conjugates (ADCs) offer a potentially new way to treat cancer in people by combining the unique targeting of mAbs with the cancer-killing ability of cytotoxic drugs, thereby allowing for sensitive discrimination between healthy and diseased tissues (Bidard and Trédan, 2014).

While passive immunotherapy holds significant potential for treating cancer, therapeutic mAbs have not yet been introduced in veterinary oncology. Several tumour-associated antigens, including CD20 (Jubala et al., 2005), epidermal growth factor receptor (EGFR) (Gama et al., 2009; Fukuoka et al., 2011; Sabattini et al., 2015), HER-2 (Ferreira et al., 2010; Singer et al., 2012), vascular endothelial

* Corresponding author. Tel.: +39 051 6751871.
*E-mail address:* marconato@centroncologicovet.it (L. Marconato).

http://dx.doi.org/10.1016/j.tvjl.2015.02.020
1090-0233/

growth factor (VEGF) (Aresu et al., 2014) or platelet-derived growth factor receptor (PDGFR) (Maniscalco et al., 2013), have been identified in canine malignancies; however, mainly for financial reasons, only a few attempts have been made to generate 'caninised' mAbs (Jeglum, 1996; Singer et al., 2014) or to evaluate humanised mAbs in veterinary oncology (Impellizeri et al., 2006).

In the 1980s, the United States Food and Drug Administration (FDA) approved the first chimeric antibody, rituximab, for the treatment of human B-cell non-Hodgkin lymphoma (Grillo-López, 2000). Rituximab represents one of the best examples for mAbs of proof-of-concept. Unfortunately, it failed to show efficacy in canine B-cell lymphoma due to the lack of homology between humans and dogs in the CD20 epitope that is recognised by rituximab (Impellizeri et al., 2006; Ito et al., 2014). In general, lack of epitope homology between human and canine proteins and consequently cross-reactivity of the mAbs for the canine protein limit their use in dogs.

In humans, many vaccines have reached Phase 2–3 clinical trials. Most paired tumour-associated antigens (TAAs) have an immune-activating adjuvant to stimulate the humoral and/or cellular immune responses against these TAAs (Schlom, 2012). In veterinary oncology, a variety of vaccines eliciting an anti-tumour immune response have been generated, involving peptides, cells, DNA, viruses and ex vivo generated dendritic cells (Bergman et al., 2003; Turek et al., 2007; Peruzzi et al., 2010; Grosenbaugh et al., 2011; Sorenmo et al., 2011; Marconato et al., 2014; Riccardo et al., 2014).

Targeted therapy and cancer vaccines have introduced new challenges for oncologists, such as determining the optimal dosing and administration schedules, and a proper understanding of the mechanism of activity of these experimental agents has become essential for the optimal design of any clinical study. In clinical trials evaluating traditional chemotherapeutic agents, toxicity is generally determined through the degree of myelosuppression and gastrointestinal (GI) side effects. Most conventional chemotherapeutic cytotoxic agents cause cell death by directly inhibiting DNA synthesis or by interfering with DNA function. For these reasons, chemotherapeutic cytotoxic agents are not tumour-specific and are thus associated with considerable morbidity. Conversely, targeted therapy and cancer vaccines usually do not cause significant toxicity. In addition, assessment of efficacy may require a paradigm shift. Effective cytotoxic chemotherapy leads to tumour volume reduction, while some targeted therapies may impart a clinical benefit by stabilising tumours rather than by shrinking them. For cancer vaccines, efficacy may be even harder to anticipate.

These new antitumour strategies challenge the existing paradigm for experimental design, conduct and analysis of Phase 1, 2 and 3 oncology clinical trials, prompting oncologists to turn to different endpoints for appropriate dosing, schedule selection, and efficacy assessment (Park et al., 2004).

In this review, the impact of targeted therapies and cancer vaccines on the design of oncology clinical trials is discussed in terms of target identification, study endpoints, and overall clinical protocol.

## Target identification

Identifying the biological origin of disease and the potential targets for therapeutic intervention is the first step in target-based drug discovery. A target-based drug discovery programme is aimed at developing drugs that selectively modulate the effects of selected genes or gene products (the therapeutic targets) without adversely affecting other vital molecular mechanisms. This involves discovering these targets, a process referred to as target identification.

Signal transduction pathways involved in cancer biology are generally investigated using sequencing techniques. For some human tumours, gene expression profiles have been proposed as potential biomarkers to predict treatment response and to identify new therapeutic targets. Human tumours may have distinct molecular subtypes and different therapeutic approaches may be required for each subtype (Bodey et al., 1996). Only recently, genomic technologies have been considered for canine tumours, and in the future these data will be validated and used for specific targeted therapy (Klopfleisch, 2015).

Different approaches may be considered for target identification. One approach is to compare the amounts of individual proteins in cancer cells with those expressed in normal cells. Proteins that (1) are over-expressed in cancer cells, (2) provide a selective advantage to tumour cells (growth or survival), and (3) are not being expressed (or expressed at much lower levels) by non-cancerous cells represent ideal targets (Zhang et al., 2009). Another approach is to determine whether neoplastic cells produce mutated proteins that participate in cancer generation and progression. Chromosomal abnormalities may also be present in neoplastic cells, resulting in fusion genes whose products may drive cancer development. Such fusion proteins may become potential targets for treatment (Cavallo et al., 2007; Iezzi et al., 2012; Aricò et al., 2014).

Even if a good therapeutic target is identified and an inhibitor of that target with in vitro or in vivo pharmacological efficacy is available, response in the clinic cannot be fully anticipated, as effectiveness of targeted therapy does not always correlate with target overexpression (Douthwaite and Jermutus, 2006). Thus, some target-positive tumours may fail to respond (primary resistance), while some target-negative tumours may show some response. It is possible that blocking the growth factor receptors may benefit certain patients even if the cancer is not overexpressing them, thereby challenging clinical trial design, particularly patient eligibility (Nicolaides et al., 2014).

## Selection of primary endpoints

In human oncology patients, overall survival (OS, i.e. the interval from randomisation to death from any cause) represents the gold standard endpoint for ascertaining the clinical benefit of (and approving) traditional anticancer treatments, as it is not subject to investigator interpretation (Williams et al., 2004). Possible challenges include crossover or subsequent therapies (which may confound the survival benefit that can be attributed to any individual drug or protocol) and the requirement for a large population and/or a long follow-up compared to other endpoints in order to show statistical differences, especially for slowly progressing cancers (Johnson et al., 2003). In veterinary oncology, OS is not the most appropriate endpoint, as it may be biased by several tumour-unrelated factors, including the owner's financial concerns.

Time-to-progression (TTP, i.e. the interval from randomisation to disease progression) represents another commonly used endpoint. Patients must be evaluated on a regular basis, and all disease sites should always be assessed. In addition, the same assessment technique should be used at each follow-up to reduce bias (Johnson et al., 2003). This clinical trial endpoint can be achieved sooner than OS and there is no confounding effect by crossover or use of second-line therapies. Nevertheless, TTP is at best only an estimate, as it may vary based on the scheduled frequency of evaluation.

Objective response rate (ORR) is also commonly used and refers to the portion of patients showing a predefined tumour size reduction (depending on the adopted response criteria, WHO [World Health Organization] vs. RECIST [Response Evaluation Criteria in Solid Tumours]) (Nguyen et al., 2013; Heller, 2015) for a minimum time period (typically 21–28 days). Response duration is measured from the initial response until documented tumour progression (Johnson et al., 2003). Like TTP, response duration can only be estimated and varies based on the frequency of assessment.

TTP and ORR may be considered ideal primary endpoints, but both can be difficult to measure in veterinary oncology, because of