Review

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Theileria-transformed bovine leukocytes have cancer hallmarks

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The genus *Theileria* includes tick-transmitted apicomplexan parasites of ruminants with substantial economic impact in endemic countries. Some species, including *Theileria parva* and *Theileria annulata*, infect leukocytes where they induce phenotypes that are shared with some cancers, most notably immortalization, hyperproliferation, and dissemination. Despite considerable research into the affected host signaling pathways, the parasite proteins directly responsible for these host phenotypes remain unknown. In this review we outline current knowledge on the manipulation of host cells by transformation-inducing *Theileria*, and we propose that comparisons between cancer biology and host–*Theileria* interactions can reveal chemotherapeutic targets against *Theileria*-induced pathogenesis based on cancer treatment approaches.

Theileria-induced bovine immune cell transformation

Of the estimated 1.2–10 million species in the phylum Apicomplexa, only ~6000 have been described [1]. Almost all are intracellular parasites of vertebrate and invertebrate hosts, but the degree of diversity amongst these species is astounding. They have complex life cycles with diverse morphologies and are distributed over much of the globe. A member of the class Hematozoa, the genus *Theileria* includes tick-transmitted parasites of wild and domestic ruminants that cause a substantial economic burden (Box 1). A single species, *Theileria parva*, is responsible for >1 million cattle deaths per year in sub-Saharan Africa, at a cost of US$ >300 million [2]. The US Government 'Feed the Future' initiative (http://feedthefuture.gov/sites/default/files/resource/files/FTF_Guide.pdf) and the reformed Committee on World Food Security [3] have focused on reducing poverty and eliminating hunger from the world. In the wake of these renewed efforts, there has been a considerable boost in funding aimed to curb the impact of *T. parva* in sub-Saharan Africa.

Theileria parasites have several characteristics that make them unique among the known apicomplexa. During tick feeding, sporozoites are inoculated into the blood and infect white blood cells where they develop into schizonts [4]. Unlike many apicomplexans, *Theileria* resides in the host cytosol instead of inside a parasitophorous vacuole. During host cell mitosis, the schizonts bind to the host mitotic spindle, ensuring segregation into both daughter cells with great efficiency to maintain the infection rate [5].

With the fates of parasite and host cell closely intertwined, some *Theileria* species have evolved mechanisms to induce proliferation, immortalization, and dissemination of the host cell [6], arguably the phenotypes that most define cancer. In this review, when discussing *Theileria* parasites, we are only referring to transformation-inducing species in their cattle hosts (see Glossary). The most thoroughly studied are *T. parva*, which transforms bovine B and T lymphocytes, and *T. annulata*, which transforms macrophages, dendritic cells, and B cells [7]. Transformation in both cases is induced during the schizont stage (Figure 1). Both *T. parva* and *T. annulata* transform B cells, but whether or not the mechanism of host cell transformation is the same in both species remains to be established. Unfortunately, many relevant studies to date used only one or the other of these species; therefore, several comparative genomic studies have assumed the mechanism of host cell transformation to be the same [8–10]. Here we follow the same premise. *T. parva* and *T. annulata* are likely to have coevolved with different buffalo species (*Syncerus caffer* for *T. parva* and *Bubalus bubalis* for *T. annulata*) because these ruminant species appear to host the most diverse parasite populations and are not known to succumb to disease upon infection [11]. However, if left untreated, these parasites can kill susceptible cattle in less than 3 weeks, with a mortality that approaches 80% in some areas [12]. The conversion of *Theileria* schizont-infected cells into immortal cell lines depends on acquired characteristics that are remarkably similar to those exhibited by some cancerous cells, such as immune evasion and resistance to apoptosis. Each of these characteristics provides an opportunity for the development and use of cancer therapies for treating *Theileria* infections, and possibly a better understanding of the molecular interactions underlying these phenotypes. The goal of this review is to outline what is known about host cell manipulation by the transformation-inducing *Theileria* in the context of hallmarks of infection that are shared with many cancers [13].

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Glossary

Autophagy: a process whereby cells degrade intracellular components to promote their own survival in response to cellular stress.
Classical dendritic cells: leukocytes that sense tissue injury, capture antigens, and present those antigens to T lymphocytes to induce immunity to foreign antigens and enforce tolerance to self-antigens.
Fas ligand: a protein that binds the Fas receptor and induces apoptosis upon binding, a mechanism used by cytotoxic T lymphocytes to induce apoptosis in target cells.
Hypoxia inducible factor 1α (HIF-1α): a transcription factor that regulates the cellular response to low oxygen conditions by activating the transcription of genes involved in energy metabolism, angiogenesis, and apoptosis.
IκB kinase complex (IKK complex): an enzyme complex consisting of three monogenic protein subunits (α, β, γ) that catalyzes the specific phosphorylation of the inhibitory IκB-α protein. IκB-α phosphorylation causes the dissociation of IκB-α from NF-κB, which then migrates to the nucleus and activates gene expression.
Interferon γ (IFN-γ): a cytokine produced mostly by T cells and natural killer cells that has antiproliferative, immunoregulatory, and proinflammatory activity during host defense.
Interleukin 2 (IL-2): a cytokine that is secreted by activated T cells and is important for lymphocyte proliferation, the clearance of self-reactive T cells, and the maintenance of regulatory T cells.
Leukocyte: white blood cells involved in immunity that circulate in the peripheral blood and consist of lymphocytes, monocytes, basophils, and neutrophils.
Lymphocyte: mononucleated leukocytes that include T cells, B cells, and natural killer (NK) cells.
Macrophage: a type of phagocytic leukocyte that differentiates from monocytes and plays crucial roles in host defense against pathogens, immune regulation, and wound healing.
Matrix metalloprotease 9 (MMP9): a secreted enzyme involved in the degradation of extracellular matrix that is crucial for homeostatic functions such as vascular development, cell migration, and wound repair.
MHC class I: a molecular complex found on nearly every nucleated cell that displays protein fragments, largely derived from the cytosol, to $CD8^+$ T cells and is crucial for the generation of antigen-specific adaptive immune responses and the inhibition of natural killer cell killing.
MHC class II: a family of molecules mostly found on professional, antigen-presenting cells such as dendritic cells, macrophages, and B cells that displays protein fragments derived primarily from extracellular proteins to $CD4^+$ T cells.
Nuclear factor κ-light chain enhancer of activated B cells (NF-κB): a conserved transcription factor protein complex canonically involved in the cellular production of cytokines and survival signals in response to stimuli such as stress and infection.
Phagocytosis: a process by which cells consume extracellular particles to form an internal vesicle containing those particles.
P53: an important tumor-suppressing transcription factor that regulates cellular responses to a myriad of cellular stressors including hyperproliferation, DNA damage, and telomere attrition.
Telomerase reverse transcriptase (*TERT*): a ribonucleoprotein enzyme that adds DNA repeats to the 3′ end of telomeric DNA at the ends of eukaryotic chromosomes.
Transformation: the modification of a eukaryotic cell to cause it to have some or all of the characteristics of a cancer cell (this is the definition used in this review and throughout the field of *Theileria* research).
Tumor growth factor β (TGF-β): a cytokine secreted by many cell types that regulates developmental programs and cell behavior by modulating cell proliferation, morphogenesis, differentiation, and tissue homeostasis and regeneration.
Tumor necrosis factor α (TNF-α): a cytokine produced by many immune and epithelial cell types that plays a central role in systemic inflammation, apoptosis, and immune system development.

Box 1. Outstanding questions and potential experimental approaches to address them

- Which parasite-derived molecules drive host proliferation? Screen parasite cDNA library for proliferation induction (e.g., carboxyfluorescein succinimidyl ester staining or cell counting).
- How do these parasites induce and maintain replicative immortality of their host cells? Quantify TERT activity in the presence or absence of NF-κB inhibitors.
- Which host genes regulate the metastasis of infected cells? Knockout known mammalian regulators of metastasis and quantify migration in a gel matrix.
- How do these parasites avoid autophagy despite host metabolic stress? Determine the effect of autophagy-inducing compounds on infection rates *in vitro* and *in vivo*.
- Which host molecules mediate contact-dependent proliferation of host cells? Screen mammalian and non-mammalian cells for an inability to support *Theileria* transformed bovine leukocyte growth.
- What is the effect of p53 sequestration on the *Theileria* surface during an infection? Alanine-scan p53 and screen for sequestration on the parasite surface.
- How do *Theileria* parasites maintain a carrier state in its bovine hosts? Determine if bTERT can be used as a vaccine in cattle.
- Do *Theileria* parasites induce genomic instability or mutations in their bovine hosts? Perform karyotyping and whole-genome sequencing of *Theileria*-transformed bovine leukocytes in comparison to their isogenic, uninfected controls.

Phenotypes *Theileria*-infected immune cells share with cancer cells

Despite many well-characterized differences among them, all cancer types share a defined set of phenotypes [13]. Several of these properties are also observed during *Theileria* infection.

Mechanisms of Theileria-induced proliferative signaling

Theileria-induced transformation leads to activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)-dependent proliferative signaling. NF-κB regulates a multitude of biological processes and is ubiquitously activated in hematological malignancies [14,15]. Pattern-recognition receptors can also activate NF-κB to induce *in vivo* antimicrobial programs that are crucial for innate and adaptive immunity [16], and many pathogens have the ability to suppress NF-κB signaling [17,18]. The IκB kinase (IKK) complex activates NF-κB, and *Theileria* schizonts have been shown to constitutively activate the IKK complex on their cell surface, possibly by *trans*-autophosphorylation [19]. Consequently, investigations into the mechanisms by which *Theileria* parasites manage to evade the immunostimulatory effects of NF-κB signaling provide an opportunity to discover novel therapeutics against *Theileria* infection.

Most healthy cells require a growth signal to undergo mitotic division as a mechanism to prevent inappropriate proliferation. For example, the multiplication of mature, naïve lymphocytes is largely regulated by antigen receptor stimulation as well as by a second, co-stimulatory signal [20]. While bovine leukocytes transformed by *T. parva* [21–24], but not by *T. annulata* [25,26], can produce interferon γ (IFN-γ) and interleukin 2 (IL-2), both produce and respond to tumor necrosis factor α (TNF-α) [27,28]. However, it has been suggested that some *Theileria*-transformed cell lines may grow independently of growth factors [29]. Cancer cells have been shown to achieve growth factor independence by (i) producing growth factor ligands themselves, (ii) sending stimulatory signals to nearby cells that provide growth factor ligands in return, (iii) becoming hyper-responsive to otherwise limiting levels of growth factor ligands, or (iv) constitutively activating downstream signaling pathways of a growth factor receptor [13]. A comparison of cell cycle-regulated genes between cancer and normal tissues revealed significant differences in proliferation programs and potential drug targets [14].

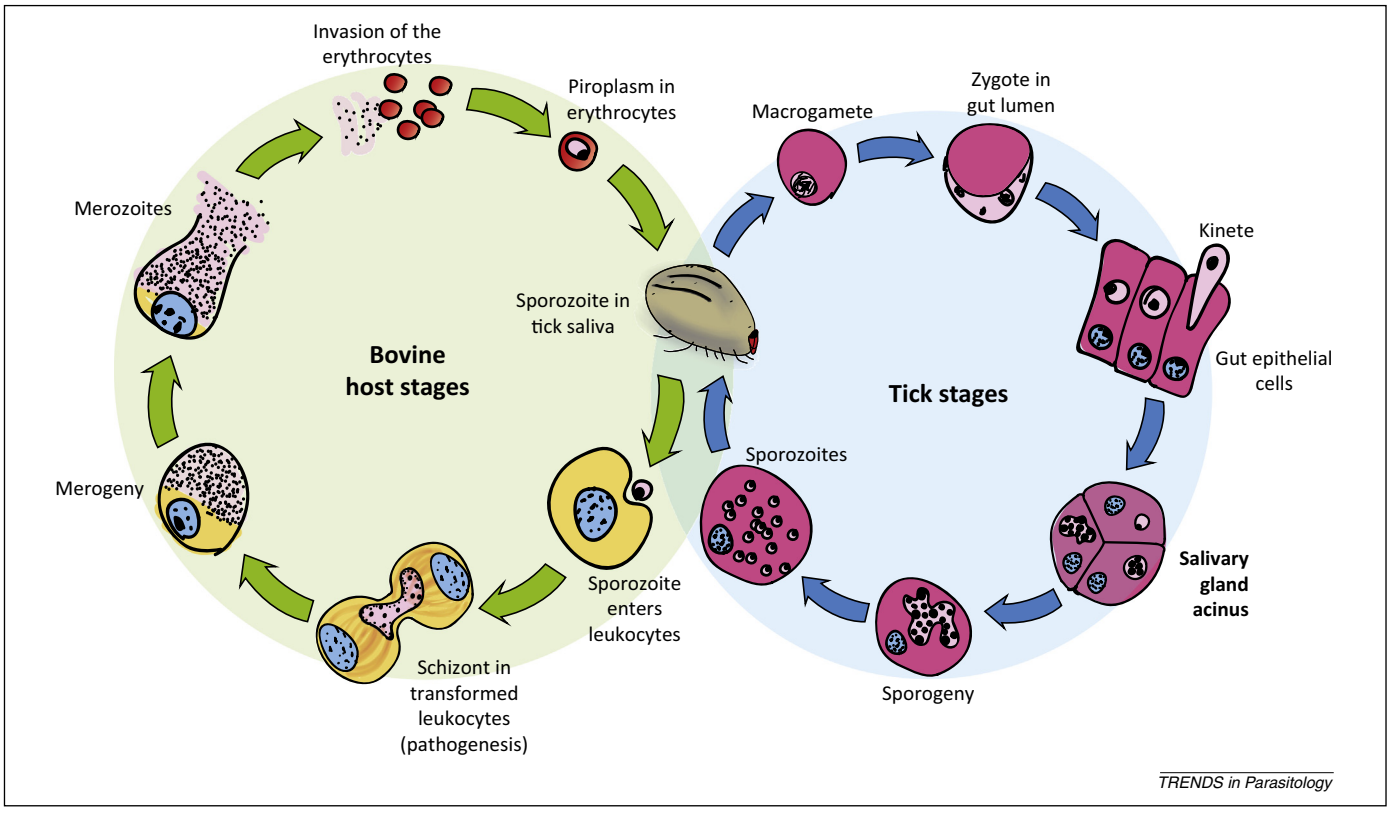

Figure 1. The life cycle of transformation-inducing *Theileria*. Parasites in the genus *Theileria* are dixenic. The definitive host is a tick (*Rhipicephalus* for *T. parva* and *Hyalomma* for *T. annulata*), which ingests piroplasms from the blood of an infected bovine host. Tick feeding can occur over 4–5 days, but engorgement takes place over a 24 h period. Even though the tick ingests millions of infective piroplasms, only a small fraction will survive to be transmitted to the next host. Ingested piroplasms that survive in the presence of gut digestive enzymes and microbiota develop into micro- and macrogametes in the gut lumen, where syngamy and, therefore, genetic recombination occurs. *Theileria* zygotes then must evade tick phagocytic cells and invade basal lamina epithelial cells, where they differentiate into the complex and motile kinete stage. During the molting of the tick, the kinete stage migrates to the salivary glands and selectively enters particular salivary gland acinar cells. Proliferation results in a sporoblast syncytium which, upon segmentation, forms the bovine-infective sporozoite stage. Tick feeding typically occurs on the bovine head, where the tick inoculates the bovine host blood with sporozoites that infect varying subsets of leukocytes, depending on the *Theileria* species – characteristically all lymphocytes for *T. parva*, and macrophages, B cells, and dendritic cells for *T. annulata*. In these cells, the *Theileria* sporozoites develop into multinucleated schizonts. Infected bovine leukocytes then migrate to the draining parotid lymph node, where they clonally proliferate and disseminate into various host tissues. This is generally believed to be the most pathogenic stage of the transformation-inducing *Theileria* life cycle. Upon some signal (temperature increase for *T. annulata* and an unknown signal for *T. parva*), the schizont will undergo cytokinesis and develop into the uninucleate merozoite stage which is infective to bovine red blood cells. Presumably, the parasite then induces host apoptosis, and the merozoites are released into the bloodstream, invade new erythrocytes, and develop again into multinucleated tick-infective piroplasms.

Because these proliferation genes are cell cycle regulated, a genome-wide transcriptome analysis of cell cycle-synchronized *Theileria*-infected cells and uninfected cells could provide an initial list of host or parasite genes that may play a role in *Theileria*-induced hyperproliferation.

Several other pathways are involved in the proliferative signaling of *Theileria*-transformed cells, although a direct link to a single parasite molecule has not been shown. These have been extensively reviewed elsewhere [15], and include mitogen-activated protein kinases, SRC family kinases, casein kinase-2, and phosphatidylinositide 3-kinase, as well as miRNA deregulation [19]. These other cancer-related affected pathways may also yield alternative chemotherapies to *Theileria* infection. For example, *T. annulata* was recently shown to secrete a prolyl isomerase called TaPIN1 that interacts with host ubiquitin ligase FBW7, which promotes host proliferation by stabilizing the transcription factor c-JUN [30]. Delineating the mechanisms by which the parasite manipulates these pathways and how they affect pathogenesis will be a crucial aspect to understanding *Theileria* host–pathogen interactions.

Theileria-induced replicative immortality

A very distinctive and enigmatic phenotype of *Theileria*-transformed cells is that they can be cultured *in vitro* indefinitely, exactly as any standard established cell line [31]. This *in vitro* phenotype may play a role in persistence *in vivo* because host survival during a natural infection or vaccination of cattle with a live *T. parva* or *T. annulata* vaccine induces a carrier state in the recipient, raising some cautionary notes regarding the potential spread of these parasite stocks associated with the movement of vaccinated cattle [16].

Most primary cells will rapidly die in culture owing in part to the gradual shortening of telomeres with each cell division. Approximately 85% of cancers surmount this barrier by increasing the activity of telomerase [17], a reverse transcriptase that enzymatically elongates telomeres *de novo*. Mechanisms of telomerase-dependent immortalization have been the target of intense study in the cancer research community. Telomerase inhibitors effectively restrict *T. parva*-induced transformation [18]. Recent work has shown that human telomerase, hTERT, can bind to the p65 NF-κB subunit and direct it to a subset of NF-κB

promoters to initiate the transcription of several genes including *hTERT* [32]. It is, therefore, tempting to speculate that constitutive activation of the IKK complex by *Theileria* parasites could induce a similar positive feedback on the expression of bovine telomerase, *bTERT*. A bTERT-targeted vaccine against *Theileria* infection is also an intriguing possibility because hTERT has been the target of vaccine trials for human cancers [33]. However, *bTERT* was not reported to be differentially expressed during infection of bovine B-lymphosarcoma cells by *T. annulata* [34,35], indicating that *Theileria* transformation may use an alternative mechanism to lengthen telomeres, or that existing telomerase may be hyperactivated.

Activation of invasion and metastasis

Theileria-transformed host cells home to the draining lymph node where they proliferate and disseminate into various organs, causing lymph node swelling, fever, anorexia, and frothy nasal discharge. In fact, pulmonary edema is often the cause of death for cattle infected with *Theileria* [12,36]. *T. annulata*-infected macrophages invade tissues via an amoeboid invasion mechanism [37], for which matrix metalloproteinase 9 (MMP-9), transforming growth factor β (TGF-β), and TNF-α are essential [27,38,39]. *Theileria* parasites have been shown to have a close association with host microtubules, which play a crucial role in metastasis [40]. *T. annulata* recruits end-binding protein 1, a crucial component of host microtubule regulation, to its cell surface via interactions with *T. annulata* polymorphic piroplasm antigen, p104 [41]. The *T. annulata* proteins TaSE (*T. annulata* secretory protein) [42] and the conserved glycosylphosphatidylinositol-anchored protein gp34 [43] also localize to host microtubules, although the precise roles of these proteins are not yet well understood.

Recent developments in genome-scale technologies have led to the description of human metastasis-suppressor genes with potential for therapy targeting [44]. Standard cancer cell invasion assays are well suited for investigating the role of the bovine orthologs of these genes in *Theileria*-induced pathogenesis [37] or for the discovery of new metastasis-suppressor genes by comparing infected bovid cells that are resistant or susceptible to *Theileria* pathogenesis [45].

Deregulation of cellular energetics

Metabolism is a key regulator of leukocyte function and fate, with functionally different cell subsets having distinct biosynthetic and energy requirements [46] that might be relevant to the manipulation of host cells by *Theileria* [47]. *Theileria* parasites are prototrophic for only three of the 20 amino acids [48], and the acquisition of other metabolites from the cytosol depletes nutrients from the host. *T. annulata* schizonts also induce a Warburg effect in host cells, defined by a shift in ATP generation from predominantly oxidative phosphorylation to glycolysis [49]. This metabolic switch is associated with a deregulation in the concentration of reactive oxygen species (ROS) and activation of the protein hypoxia-inducible factor 1α (HIF-1α) [50]. This may represent the need of the host cell to survive despite nutrient depletion by the parasite, or active manipulation by the parasite to bolster host cell proliferation. The targeting of ROS, HIF-1α, or glycolysis in *T. annulata*-transformed cells reverses this effect, exactly as in certain cancers [51,52]. In some cancers, genetic alterations in the genes *TP53*, *MYC*, and *PI3K* play a role in the induction of a Warburg effect [53]. Because these host proteins are manipulated by *Theileria* parasites, they provide starting points for further study of the Warburg effect during infection.

Cells can survive metabolic stress by the induction of autophagy, a process which not only allows degradation and recycling of cellular components, but also leads to signaling that inhibits cell death, inflammation, and DNA damage [54]. However, autophagy could result in the clearance of *Theileria* parasites from the host cytosol [55]. Because defects in autophagy have been associated with increased tumorigenesis in some cancers [54], determining how *Theileria* parasites avoid autophagic clearance, perhaps by directly blocking autophagy [55], without inducing metabolic stress-induced cell death could provide key insights into the coregulation of autophagy and metabolism.

Inhibition of cell death in Theileria-infected cells

A crucial strategy that many intracellular protozoan parasites use to ensure advancement into the next stage of their life cycle, as well as to ensure transmission between hosts, is to block apoptosis of the infected host cell, prolonging its life [56]. *Theileria* schizonts induce anti-apoptotic proteins such as cellular FLICE-like inhibitory protein (cFLIP) and cellular inhibitor of apoptosis proteins (cIAPs) [25] by activating host IKK complexes [57] and upregulating or maintaining high c-MYC expression [34,58]. *Theileria* can also inhibit host pro-apoptotic signaling by sequestering host p53 [59] on their cell surface via unknown receptor(s). *T. parva* infection also confers resistance to Fas/FasL-induced apoptosis [60], which might be crucial for the evasion of cytotoxic T lymphocytes (CTLs) [61] and activation-induced cell death [62]. Investigations into how *Theileria* parasites evade activation-induced cell death and CTL-mediated killing could lead to insights that improve vaccine development and to therapeutics for protection against many pathogens and some cancers [63].

Immune evasion and inflammation in Theileria-infected cells

Despite *Theileria* immune evasion, live vaccines against *Theileria* parasites have had some success. Vaccinations with cocktails of live parasites can provide reasonable cross-protection against disease [64]. For *T. parva* [65] and *T. annulata* infection [66], there is evidence that MHCI-restricted $CD8^+$ T cells mediate protective immune responses. Antigen variation is thought to play a role in immune evasion at the population level, inducing a very restricted CTL response [67,68].

Immune evasion may also involve modulation of the host immune response. Even though possible molecular mechanisms are unclear, some patterns are emerging. *T. annulata*-infected bovine macrophages downregulate some macrophage markers and lose functions such as Fc-mediated phagocytosis and the production of antimicrobial

molecules, including nitric oxide and TNF-α [69]. They also upregulate several cytokines that are known to play a crucial role in immune responses against parasites [70,71]. However, there is evidence that inflammatory cytokine production (e.g., IFN-γ) is somehow delayed by the parasite *in vivo* until after schizont development, when signaling is not as effective [72]. This may be one reason why *T. annulata* is not cleared by a Th1 response, even though this is typically the case for other macrophage-resident protozoan infections [70,73].

While *T. annulata*-infected macrophages are efficient antigen-presenting cells [74], they are also able to induce memory-independent proliferation of autologous αβ and γδ T cells from naïve donors *in vitro* in a manner similar to a superantigen [75]. During *in vivo T. annulata* infection, activated T cells migrate from the lymph nodes to the efferent lymph, where an antiparasite response is not required, and downregulate CD2, a crucial adhesion molecule for cytolytic activity [76]. Unlike *T. parva*, and perhaps due to this misdirected T cell response, it has not been possible to isolate *T. annulata*-specific T cell lines from infected cattle [73].

T. parva-infected lymphocytes can also upregulate several immunoregulatory molecules, including IFN-γ and IL-2, both of which improve the transformation efficiency of host lymphocytes [22,23]. Because MHC I is essential for *T. parva* invasion of bovine lymphocytes [77], and IFN-γ is known to upregulate MHC I [78], the expression of IFN-γ is an apparent mechanism by which the parasite can increase the susceptibility of circulating lymphocytes to infection, and proliferative cytokines likely aid the division of parasitized cells. However, cytokine profiles often vary among *T. parva*-infected bovine T cell clones [79]. One cytokine that has been consistently associated with *T. parva* infection is IL-10, which may have significant immunoregulatory roles during infection [79], although these roles are not well defined.

Interestingly, *T. parva*-parasitized cells constitutively express MHC class II (MHC II) molecules on their surface that have a higher molecular mass than those of uninfected cells [80]. Because MHC class II molecules have conserved *N*-linked glycosylation sites, post-translational modifications could play a crucial, novel role in regulating immune responses to these pathogens [81].

Despite the facts that *T. parva* infects all subsets of B and T cells with varying effects on the pathogenicity of infection [82], and that there is an expansive literature and taxonomy of T cell subsets, the effect of *T. parva* infection on the differentiation of infected and uninfected T cells *in vivo* is not well known. Pathogens and tumors have also been known to use other immune evasion strategies, such as dormancy, sequestration, failure of antigen display, and antigenic variation [83]. Investigations into these potential mechanisms of immune evasion in cattle and buffalo could lead to insights that are crucial for the improvement of vaccine regimens.

Differences between *Theileria*-infected bovine cells and cancer cells

In contrast to the phenotypes already reviewed, *Theileria*-transformed bovine cell proliferation may lack some of the characteristics of cancer cells. Alternatively, the relevant evidence may have yet to be uncovered. Two such phenotypes are the evasion of growth suppression and a breakdown in genomic integrity.

Evasion of growth suppression

Most somatic cells stop proliferating at a specific density as a result of interactions with other cells, a feature known as contact inhibition. Cancer cells must overcome this barrier to proliferation to grow [84]. In contrast to both normal somatic cells and many cancer cells, *T. parva*-transformed lymphocytes require contact with other infected or uninfected cells to proliferate [85]. The expression levels of known regulators of contact inhibition, Merlin or liver kinase B1, are not reportedly regulated by *T. annulata* during infection [34,35], suggesting that *Theileria*-infected cells may achieve contact independence via an as-yet undiscovered mechanism.

Despite its well-known role as a growth suppressor, in some late-stage tumors TGF-β signaling can induce a context-dependent cellular program that enhances metastasis as a result of mutations that either inhibit core TGF-β signaling components, or regulate tumor-suppressor signaling components downstream of TGF-β receptor signaling (e.g., increased MYC, decreased nuclear p53) [86]. *T. annulata*-transformed leukocytes appear to induce the latter mechanism because TGF-β signaling drives metastasis while altering transcript levels of many TGF-β-regulated genes [39], whereas the core signaling components have mostly unaffected expression levels [87]. *Theileria* parasites are also known to induce the expression of host MYC [34,58], and sequester host p53 in the cytosol [59] to prevent host apoptosis, and these proteins may therefore also play a role in regulating host cell invasiveness.

Genomic integrity

With all of the hallmarks that *Theileria* transformation has in common with many kinds of cancers, the question remains as to whether or not these parasites actually induce cancer in host cells. As stated by Vogelstein and Kinzer [88]: 'Cancer is, in essence, a genetic disease'; consequently, to properly answer this question it must be resolved whether or not these parasites induce genomic mutations in their host. There is evidence that *Theileria* infection imposes some irreversible effects on host cells. For example, an established bovine leukemia cell line infected with *T. annulata* and then chemically cleared of the parasite exhibits some irreversible gene expression changes and, eventually, dies from apoptosis within a couple of days [34,38]. The cytokine profiles of infected cells also vary considerably among T cell clones, even within T cell subsets [79], indicating that *Theileria* parasites may cause stochastic effects on their host cells.

Although there are no reports of genomic instability in *Theileria*-transformed cells, there is evidence that *Theileria* parasites and other apicomplexans such as *Cryptosporidium* and *Toxoplasma* affect host DNA integrity [89]. *Theileria* parasites have been shown to sequester p53 on their surface, in the host cytosol, presumably preventing it from executing its role in maintaining genomic stability [59]. The p53 negative regulator

MDM2 (mouse double minute 2 homolog) is also upregulated in *T. parva*-infected cells [18]. In addition, infected host cells upregulate miR-155 upon infection, and this could induce genome instability by downregulating genes involved in DNA repair [90]. *T. annulata* transformation also upregulates host SMYD3 (SET and MYND domain containing 3), a histone 3 lysine 4 methyltransferase that plays a role in transformation [38]. Methylation has been shown to have an essential role in maintaining genome integrity [91], as well as tumor-suppressor gene inactivation [92]. Therefore, SMYD3 may impact on host genome integrity as a result of *Theileria* transformation.

Multigene families

A mesmerizing question persists in the study of *Theileria*–host interactions: which parasite molecules (proteins, lipids, RNA, other) are required for, or contribute to, these cancer-like phenotypes? Because these parasites induce an acute infection in cattle, one might expect that interactions with host signaling 'hubs' play a crucial role in transformation, such as NF-κB [93]. Scale is a primary reason why this matter remains unresolved: while viral genomes tend to be $\sim 10^3$ to 10^5 base pairs in size, and encode tens to hundreds of genes, *Theileria* genomes are close to 10^7 base pairs in length, and have ~4000 genes, approximately half of which still have no predicted function. However, evidence is slowly accruing that two secreted multigene families, SVSP (sub-telomere-encoded variable secreted protein) and TashAT (*Theileria annulata* schizont AT-hook protein), play a role in host–parasite interactions.

The SVSP family is the largest gene family in both *T. parva* and *T. annulata*, and has been suspected of playing a role in immune evasion, given that the genes have a sub-telomere localization, are under positive selective pressure, have extensive nucleotide and length diversity, and atypical codon usage [94]. Most SVSP proteins have predicted secretion signals, and immunofluorescence studies of an individual SVSP protein showed expression in only a small percentage of *T. parva* parasites [95], reminiscent of what has been found for other telomeric multigene families involved in immune evasion [96]. However, one has been shown to localize to the host nucleus, suggestive of a potential role in host transformation [95].

TashAT-family proteins are secreted by the parasite, some of which localize to the host nucleus, and one has been found to alter the expression of the IFN-inducible bovine gene *ISG15* [97]. These proteins display a high degree of sequence conservation in their DNA-binding domains in *T. annulata*. These domains have not, however, been found in their *T. parva* homologs. It is unclear whether this implies a functional divergence of these proteins between *T. parva* and *T. annulata*, or if these domains are not functionally important for the cancer-like phenotypes of *Theileria*-transformed leukocytes [94].

Concluding remarks

Theileria parasites bind the host mitotic spindle to maintain an approximately 1:1 host-to-parasite ratio, and the proliferation of infected cells is rapid and unchecked. Therefore, it is not surprising that some *Theileria* species have evolved mechanisms to directly modify host signaling pathways similarly to some cancers [34,35]. We propose that each of the hallmarks that *Theileria* transformation shares with some cancer cells represents an opportunity for insights into host pathogenesis (Box 1) and potentially new therapeutic approaches for parasite infection. In fact, this approach is already producing results [54].

Looking forward, investigations into *Theileria*-induced pathogenesis could yield insights into the basic biology underlying cancer hallmarks. Several characteristics of the model lend itself to this use (Table 1). In fact, the use of *Theileria*-transformed bovine cells to study these phenotypes has already begun, with *Theileria*-transformed bovine cells being used as a xenograft model in mice [98]. Using standard cancer biology assays, *in vitro* systems have been developed as well [19]. The fact that no genomic instability has been reported in infected cells may indicate an absence of the confounding effects of the 99.9% bystander mutations that are found in most cancer cell lines [99]. There are *Theileria* parasites that vary in their pathogenic potential, and host cell types that differ in susceptibility, providing excellent natural experiments for investigations into parasite-induced phenotypes. Moreover, many species of *Theileria* do not seem to induce cancer hallmarks in their host cells, and hence provide an excellent opportunity for comparative investigations

Table 1. Advantages and limitations of *Theileria* infection as a model to study the cellular mechanisms that underlie cancer phenotypes

Property of *Theileria* infection model	Biological effect	Experimental design
Advantages		
No reported genomic instability	No confounding mutations owing to lack of bystander mutations (usually 99.9% of all mutations in cancer)	Parasite-affected pathways are more likely to be relevant to transformation and pathogenesis
Entirely parasite-dependent host malignancy	All phenotypes induced by a defined parasite-encoded interactome	Chemically cleared and uninfected cells can be used as isogenic controls
Existence of host cells of varying degree of susceptibility to pathogenesis	Cattle and buffalo *Theileria*-transformed cells may differ in their cancer hallmarks	Cattle and buffalo cells can be used as a comparative model of pathogenesis *in vitro* and *in vivo*
Limitations		
5% of human genes have no homologs in the *Bos taurus* genome	Some molecular mechanisms of *Theileria* transformation may be bovine-specific	The generality of all observations in this model should be investigated
Cellular products are host- and parasite-derived	Even 'clearance' of infection results in parasite cellular components in the host cytosol	Careful consideration of reagents/antibodies/probes is required

Table 2. Potential chemotherapeutic targets of cancer-like phenotypes in *Theileria*-transformed leukocytes

Cancer hallmark	Potential targets
Proliferative signaling	NF-κB or TaPIN1
Enabling replicative immortality	TERT
Activating invasion and metastasis	MMP-9/TNF-α
Deregulating cell energetics	HIF1α
Evading growth suppression	TGF-β
Resisting cell death	IAP
Immune evasion and inflammation	TERT vaccine
Genomic integrity	MDM2

into the proliferative phenotype of host cells. Only recently have parasite effector proteins been identified, the most effective method being the screening of parasite cDNA libraries in bovine B cell lines [55]. However, bioinformatics-based approaches have garnered recent attention [30] and will probably remain an important part of this field. Future *Theileria* research should focus on questions about each cancer hallmark exhibited by *Theileria*-transformed leukocytes (Table 2), and take advantage of the vast trove of resources and insights developed by cancer biologists over the years.

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