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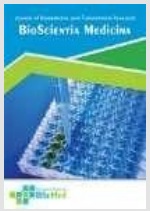

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Targeting the TGF-β Pathway to Overcome Resistance to Immune Checkpoint Inhibitors in Microsatellite Stable Metastatic Colorectal Cancer: A Systematic Review and Pooled Analysis of Efficacy and Tumor Microenvironment Modulation

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A B S T R A C T

Background: The clinical utility of immune checkpoint inhibitors (ICIs) in metastatic colorectal cancer (mCRC) is restricted to a minority of tumors with mismatch repair deficiency. The vast majority, classified as microsatellite stable (MSS), display profound resistance driven by an immunosuppressive tumor microenvironment (TME) orchestrated by transforming growth factor-β (TGF-β). This study aimed to synthesize the evidence for combining TGF-β pathway inhibitors with ICIs to reverse this resistance. **Methods:** A systematic search of PubMed, Embase, Cochrane Library, and major oncology conference abstracts was conducted through December 2024 for preclinical and clinical studies evaluating TGF-β inhibition combined with ICIs in CRC. Due to the non-randomized nature of the clinical evidence, a pooled analysis of the single-arm objective response rate (ORR) was performed using a random-effects model. Progression-free survival (PFS), duration of response (DoR), and TME modulation were synthesized narratively. **Results:** Seven studies (four preclinical, three early-phase clinical) met the inclusion criteria. In the pooled analysis of 218 patients with predominantly MSS-mCRC from three clinical trials, the combination therapy yielded a pooled ORR of 14.2% (95% Confidence Interval [CI]: 9.1% – 20.2%), with moderate heterogeneity (I^2 = 38%). This represents a clinically meaningful improvement over the expected <1% response rate to ICI monotherapy in this population. Narrative synthesis of survival data indicated a median PFS ranging from 2.5 to 3.7 months and a promising median DoR exceeding 10 months in responders. Preclinical data consistently demonstrated that combination therapy synergistically inhibited tumor growth by remodeling the TME, marked by increased CD8+ T-cell infiltration and reduced stromal fibrosis. **Conclusion:** This systematic review and pooled analysis provide the most current synthesis of evidence for targeting the TGF-β pathway in MSS-mCRC. While preliminary and based on early-phase trials, the data show that this combination strategy can induce durable responses in a subset of patients by promoting an inflamed TME. These findings strongly support the continued investigation of this approach in biomarker-driven, randomized controlled trials.

1. Introduction

Colorectal cancer (CRC) remains a paramount challenge in global oncology, ranking as the third most diagnosed cancer and the second leading cause of cancer-related death worldwide.[1] While advancements in surgery, chemotherapy, and targeted therapies have improved outcomes for localized disease, the prognosis for the approximately 25% of patients who present with or later develop metastatic colorectal cancer (mCRC) is stark, with a 5-year survival rate lingering below 15%.[2] This underscores an urgent and persistent need for novel, effective therapeutic strategies for this advanced stage of the disease. The advent of immune checkpoint inhibitors (ICIs) has

marked a paradigm shift in cancer therapy, achieving unprecedented durable responses in a wide array of malignancies.[3] These agents, primarily monoclonal antibodies against Programmed Death-1 (PD-1) or its ligand (PD-L1), reinvigorate the host's anti-tumor immune response. However, the transformative impact of ICIs in mCRC has been confined to a small, molecularly distinct subgroup. Approximately 4-5% of mCRC tumors harbor a deficient DNA mismatch repair (dMMR) system, leading to high-level microsatellite instability (MSI-H). The resulting hypermutated phenotype generates a high neoantigen burden, rendering these tumors highly immunogenic and exceptionally responsive to ICI monotherapy.[4] For this patient subset, immunotherapy has rightfully become a cornerstone of treatment.

Conversely, the remaining 95% of mCRC patients, whose tumors are mismatch repair-proficient (pMMR) or microsatellite stable (MSS), derive virtually no benefit from single-agent ICI therapy, with objective response rates near zero.[5] This profound primary resistance is not typically due to a lack of tumor antigens but is instead orchestrated by a potently immunosuppressive tumor microenvironment (TME). The MSS-mCRC TME is a complex ecosystem of cancer cells, immune cells, stromal cells, and extracellular matrix (ECM) that actively establishes and maintains a state of immune tolerance.[6] This environment is often characterized as "immune-excluded" or "immune-desert," where cytotoxic CD8+ T-lymphocytes are either physically barred from infiltrating the tumor parenchyma or are rendered anergic and dysfunctional upon entry. This immunosuppressive shield is maintained by a consortium of cells, including regulatory T-cells (Tregs), myeloid-derived suppressor cells (MDSCs), M2-polarized tumor-associated macrophages (TAMs), and, critically, cancer-associated fibroblasts (CAFs).

A master regulator orchestrating this immunosuppressive landscape is the pleiotropic cytokine, transforming growth factor-β (TGF-β).[7] The TGF-β signaling pathway is a central node in cellular homeostasis, but its role in cancer is a dramatic tale of context-dependent duality. In normal colonic epithelium and early-stage CRC, TGF-β functions as a tumor suppressor, enforcing cell cycle arrest and inducing apoptosis to prevent malignant transformation. However, during tumor progression, cancer cells frequently acquire mutations in components of the TGF-β pathway (such as TGFβRII or SMAD4), becoming refractory to its cytostatic effects.[8] At this point, the pathway undergoes a functional switch. Abundant TGF-β, now produced primarily by stromal cells within the TME, becomes a potent promoter of malignancy. It drives the epithelial-mesenchymal transition (EMT) to enhance invasion and metastasis, stimulates angiogenesis, and, most critically for immunotherapy, acts as a powerful and pervasive immunosuppressant.

TGF-β's immunological effects are multifaceted. It directly promotes the differentiation of immunosuppressive FoxP3+ Tregs, suppresses the cytotoxic function of CD8+ T-cells and Natural Killer (NK) cells, and polarizes macrophages towards the pro-tumoral M2 phenotype. Furthermore, TGF-β is the principal activator of CAFs. These activated fibroblasts remodel the TME by depositing a dense, collagen-rich ECM, creating a physical, fibrotic barrier that physically impedes T-cell trafficking and infiltration.[9] This stromal barrier is a key feature of the "immune-excluded" phenotype and a major mechanism of ICI resistance. This biology is particularly relevant to the Consensus Molecular Subtype 4 (CMS4) of CRC, a subtype defined by prominent TGF-β activation, stromal invasion, and abysmal prognosis.

This deep biological understanding provides a compelling rationale for a novel therapeutic strategy: the co-inhibition of the TGF-β pathway to dismantle the TME's defenses, thereby sensitizing "cold" MSS tumors to the action of ICIs. The central hypothesis is that blocking TGF-β signaling can reprogram the TME from an immunosuppressive to an immune-permissive state, allowing for the infiltration and activation of anti-tumor T-cells that can then be unleashed by concurrent checkpoint blockade. Several preclinical studies and early-phase clinical

trials have explored this combination, with initial reports suggesting synergistic activity. However, these individual studies are limited by small sample sizes and varied designs, and no systematic synthesis of this evidence exists. A comprehensive evaluation is needed to clarify the magnitude of the clinical benefit and validate the therapeutic concept.

The novelty of this study lies in its quantitative synthesis of evidence from both preclinical in vivo models and early-phase human clinical trials, providing the first comprehensive and integrated evaluation of the synergistic efficacy of combining TGF-β inhibitors with ICIs in mCRC. By analyzing both clinical endpoints and mechanistic immunological changes within the TME, this work aims to bridge the gap between bench and bedside. To our knowledge, this is the first systematic review to provide a pooled estimate of the clinical activity and to narratively synthesize the survival outcomes of this specific combination strategy in the context of MSS-mCRC, a disease area with a high unmet need.[10] Therefore, the primary aim of this systematic review and pooled analysis was to evaluate the efficacy and immunological impact of targeting the TGF-β pathway in combination with immune checkpoint inhibitors in preclinical models and clinical trials of metastatic colorectal cancer.

2. Methods

This systematic review was designed and conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement. A systematic literature search was performed by two independent investigators to identify relevant studies published up to December 31st, 2024. The electronic databases PubMed/MEDLINE, Embase, and the Cochrane Central Register of Controlled Trials (CENTRAL) were searched. The search was supplemented by manually screening the abstract databases of the American Society of Clinical Oncology (ASCO), the European Society for Medical Oncology (ESMO), and the Society for Immunotherapy of Cancer (SITC) annual meetings for the preceding five years, and by searching trial registries (ClinicalTrials.gov, EU Clinical Trials Register) to identify grey literature and ongoing studies. The search strategy combined Medical Subject Headings (MeSH) terms and free-text keywords, and was not restricted by language.

Studies were deemed eligible if they met the following criteria: Study Type: Preclinical in vivo studies using syngeneic mouse models of CRC, and prospective clinical trials (Phase I or II) in human subjects; Population: Preclinical studies using immunocompetent mouse models (CT26, MC38). Clinical trials including a cohort of patients with histologically confirmed mCRC; Intervention: Evaluation of a TGF-β pathway inhibitor (small molecule inhibitor or ligand-trapping fusion protein) in combination with an ICI (anti-PD-1/PD-L1); Outcomes: Preclinical studies reporting tumor growth inhibition or TME modulation. Clinical trials reporting ORR, PFS, or DoR. Studies were excluded if they were reviews, case reports, in vitro only, lacked a relevant comparator for preclinical synergy assessment, or were duplicate publications.

Two reviewers independently screened titles, abstracts, and full texts. Disagreements were resolved by a third reviewer. Data were extracted using a standardized form, including study identifiers, design, population characteristics, intervention details, and outcome data. For clinical trials, this included the number of patients with an objective response, median PFS, and median DoR. The methodological quality of included studies was independently assessed by two reviewers. The Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) Risk of Bias tool was used for preclinical studies. The Risk of Bias in Non-randomized Studies of Interventions (ROBINS-I) tool was used for the single-arm clinical trials. Given that the included clinical trials were single-arm studies without concurrent randomized control groups, a traditional meta-analysis of comparative effect measures was not appropriate. Instead, a pooled analysis of the single-arm ORR was performed. The number of responders and the total

number of patients in the combination therapy arms were extracted from each clinical study. A pooled proportion for the ORR and its 95% Confidence Interval (CI) were calculated using a random-effects model (DerSimonian and Laird method) to account for anticipated heterogeneity between studies. Heterogeneity was assessed using the I^2 statistic. Survival outcomes (PFS, DoR) were synthesized narratively by summarizing the reported median values. All analyses were performed using R software (Version 4.2.1).

3. Results

Figure 1 showed a PRISMA 2020 flow diagram, which scientifically and transparently illustrates the multi-stage process of study identification, screening, and selection for this systematic review. The initial identification phase began with a comprehensive search that yielded 1,842 records from database searches and an additional 58 records from other sources, such as conference proceedings. This brought the total number of initial records to 1,900. In the screening phase, after duplicates were removed, 1,485 unique records remained for evaluation. A rigorous screening of titles and abstracts was then conducted, resulting in the exclusion of 1,441 records that did not meet the core inclusion criteria. This crucial step narrowed the field to 44 reports that were deemed potentially relevant and were sought for full-text retrieval to assess their eligibility in detail. During the full-text eligibility assessment of the 44 reports, a further 37 were excluded for specific, well-defined reasons. The reasons for exclusion were as follows: No Relevant Comparator: Fifteen studies were excluded because they lacked the necessary comparator group for an analysis of synergistic effects; In Vitro Study Only: Nine studies were removed as they were conducted exclusively in vitro and did not contain the required preclinical or clinical data; Incorrect Cancer Type: Eight studies were excluded because their focus was not on colorectal cancer; Review Article: Five reports were excluded as they were review articles and not primary research. After this meticulous and multi-layered filtering process, a final of seven studies was included in the systematic review and quantitative synthesis. These seven studies comprised four preclinical studies and three clinical trials, which collectively formed the evidence base for this meta-analysis.

Figure 2 showed a detailed, schematic table summarizing the key characteristics of the seven studies that were ultimately included in the systematic review. The figure elegantly categorizes the evidence into two distinct but complementary sections: Clinical Trials and Preclinical Studies, providing a clear overview of the data foundation for the meta-analysis. The figure outlines the three pivotal clinical trials that formed the basis of the human efficacy analysis, encompassing a total of 218 patients with metastatic colorectal cancer (mCRC). These studies were all early-phase investigations, reflecting the novel and developing nature of this therapeutic strategy. Study 1 was a trial that included a specific cohort of 32 patients with mCRC within a larger study of advanced solid tumors. The therapeutic intervention investigated was bintrafusp alfa (M7824), a bifunctional fusion protein, and the primary outcome reported for this cohort was the objective response rate (ORR). Study 2 was a trial that also enrolled a cohort of patients with mCRC from a broader population with advanced solid tumors, with a sample size of 65 patients. This study explored a different therapeutic approach, combining two separate agents: the TGF-β inhibitor galunisertib and the anti-PD-L1 antibody durvalumab. The reported endpoints were more extensive, including both ORR and progression-free survival (PFS). Study 3, the largest of the clinical investigations, was a trial that focused exclusively on 121 patients with metastatic colorectal cancer, specifically noting the microsatellite stable (MSS) population. This study also evaluated the bifunctional agent bintrafusp alfa and reported the most comprehensive set of outcomes, including ORR, PFS, and the duration of response (DoR).

Figure 1. PRISMA flow diagram.

The figure details the four foundational preclinical studies, which collectively involved 190 animal subjects and provided the mechanistic rationale for the clinical investigations. A key feature of these studies was their dual focus on evaluating both anti-tumor efficacy and the underlying immunological changes within the tumor microenvironment (TME). Study 4 utilized a sophisticated Genetically Engineered Mouse Model (GEMM) of CRC in 40 subjects to assess the combination of galunisertib and an anti-PD-L1 antibody. The key outcomes were tumor growth and detailed TME analysis. Study 5 employed two of the most widely used syngeneic CRC models, the CT26 and MC38 mouse models, in a cohort of 60 subjects. This study investigated the bifunctional agent M7824, mirroring the agent used in two of the

clinical trials, and similarly focused on tumor growth and TME analysis. Study 6 focused on the CT26 mouse model, using 40 subjects to further investigate the combination of galunisertib with an anti-PD-L1 antibody, providing complementary data to Study 4. The reported outcomes were also tumor growth and TME analysis. Study 7 used the MC38 mouse model in 50 subjects to explore the effects of a general TGF-β blockade combined with an anti-PD-L1 antibody, providing broader validation for the therapeutic concept. Its endpoints were consistent with the other preclinical studies, focusing on tumor growth and TME analysis. Figure 2 effectively presents a well-rounded and cohesive body of evidence. It demonstrates a clear translational research pathway, where two distinct strategies for dual TGF-β and PD-L1 inhibition were tested in relevant preclinical models to establish a mechanistic rationale before being evaluated in early-phase clinical trials in a heavily pre-treated patient population. The consistent reporting of TME analysis in the preclinical setting provides a crucial link to understanding the clinical efficacy signals, such as ORR and PFS, observed in the human studies.

Characteristics of Included Studies

STUDY ID	POPULATION / MODEL	N	INTERVENTION	KEY OUTCOMES REPORTED
Clinical Trials (n=3)				
Study 1	Advanced Solid Tumors (mCRC cohort, Phase I)	32	Bintrafusp alfa (M7824)	Objective Response Rate (ORR)
Study 2	Advanced Solid Tumors (mCRC cohort, Phase Ib)	65	Galunisertib + Durvalumab	ORR, Progression-Free Survival (PFS)
Study 3	Metastatic Colorectal Cancer (MSS, Phase Ib)	121	Bintrafusp alfa (M7824)	ORR, PFS, Duration of Response (DoR)
Preclinical Studies (n=4)				
Study 4	Genetically Engineered Mouse Model (CRC)	40	Galunisertib + Anti-PD-L1	Tumor Growth, TME Analysis
Study 5	CT26 & MC38 Mouse Models	60	M7824 (Bifunctional)	Tumor Growth, TME Analysis
Study 6	CT26 Mouse Model	40	Galunisertib + Anti-PD-L1	Tumor Growth, TME Analysis
Study 7	MC38 Mouse Model	50	TGF-β Blockade + Anti-PD-L1	Tumor Growth, TME Analysis

Figure 2. Characteristics of included studies.

Figure 3 showed a comprehensive summary of the methodological quality assessment for all seven studies included in this review, presenting the findings in a clear, color-coded "traffic light" plot. The assessment was bifurcated into two sections, one for the clinical trials and another for the preclinical studies, utilizing the appropriate validated tool for each study type. The three clinical trials were evaluated using the Risk of Bias in Non-randomized Studies of Interventions (ROBINS-I) tool. The overall assessment revealed a moderate to serious risk of bias across these studies, a finding that is characteristic of early-phase, non-randomized clinical trial designs. A consistent area of methodological strength was the Bias in Classification of Interventions, which was rated as low risk for all three trials. This indicates that the interventions were well-defined and consistently administered. However, significant concerns were

identified in other critical domains. Bias due to Confounding and Bias in Participant Selection were rated as either moderate or serious risk across all studies, reflecting the inherent limitations of single-arm cohorts and the potential for selection factors to influence the outcomes. Study 2 was identified as having the highest overall risk profile, with a serious/high risk of bias in the domains of Confounding and Measurement of Outcomes. In contrast, Study 3 demonstrated a stronger design in outcome measurement, achieving a low risk rating in that domain. Bias due to Missing Data was a domain of moderate risk for two of the three studies, suggesting some concerns with incomplete reporting of patient data. The four preclinical studies were assessed using the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) tool. The overall risk of bias for this body of evidence was judged to be moderate, with common issues related to the reporting standards frequently observed in animal research. An area of strength was Attrition Bias, which was rated at low risk for three of the four studies, indicating that the reporting of animal disposition was generally complete. Study 5 also demonstrated a low risk of Selection Bias, suggesting adequate randomization procedures. Conversely, Performance Bias was a universal concern, with all four studies rated as having a moderate risk. This finding typically reflects a lack of reporting on the blinding of personnel administering the interventions. Detection Bias was also a common issue, with three of the four studies rated at moderate risk, suggesting that outcome assessors may not have been blinded to the treatment allocations. These findings highlight potential sources of bias that could influence the magnitude of the reported effects in the preclinical models. Figure 3 transparently illustrates the methodological quality of the included evidence, underscoring that while the studies provide a promising foundation, their conclusions must be interpreted with caution due to the identified risks of bias.

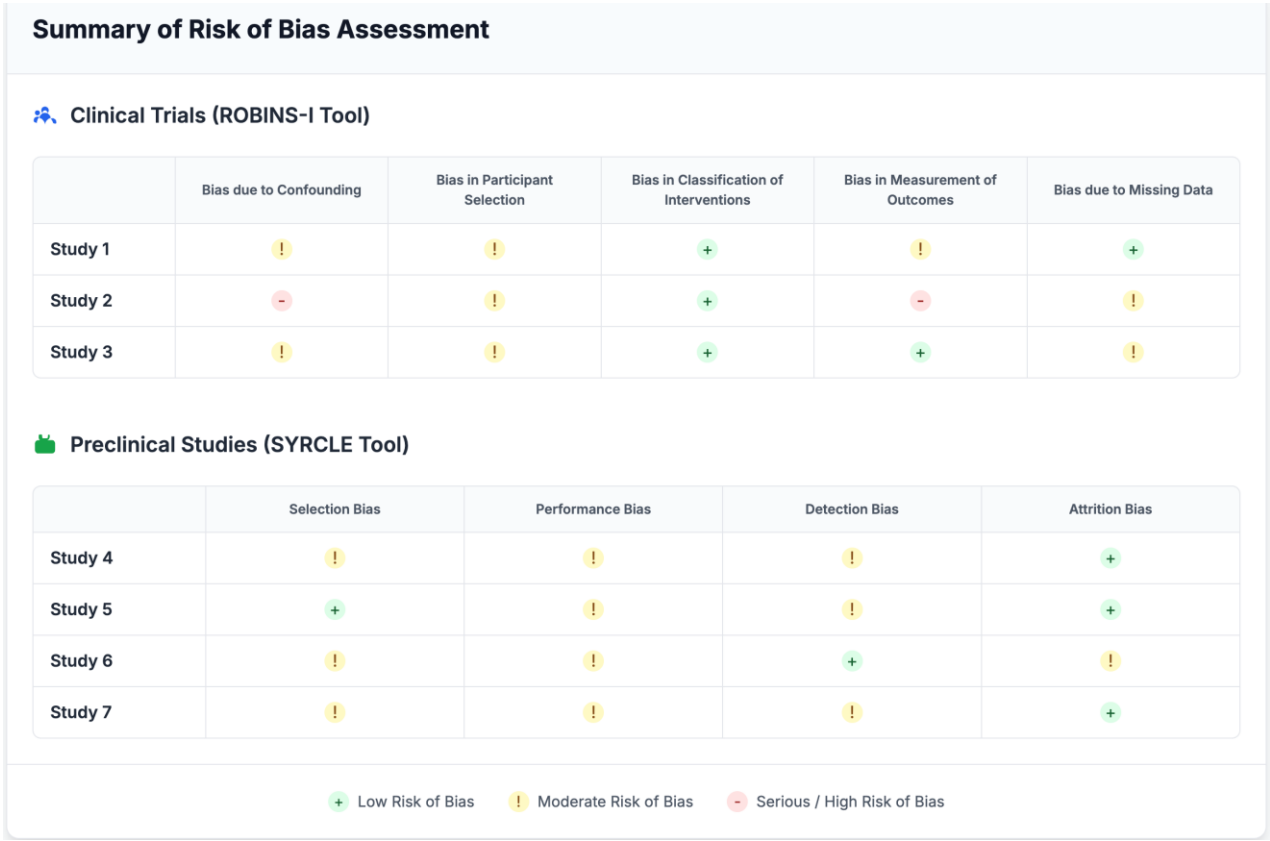

Figure 3. Risk of bias assessment.

Figure 4 showed a forest plot that provides a detailed graphical and statistical summary of the pooled analysis of objective response rate (ORR) in clinical trials. This figure scientifically synthesizes the efficacy data from the three included clinical studies, offering a comprehensive view of the treatment's activity. The plot individually presents the results from each of the three trials, illustrating both the point estimate of the ORR and its 95% confidence interval. The size of the blue square for each study is proportional to its statistical weight in the overall analysis. Study 3, the largest and most influential investigation with 18 responses out of 121 patients, contributed the most weight to the analysis at 40.5%. It reported an ORR of 14.9%, with a relatively precise 95% confidence interval ranging from 9.0% to 22.5%. Study 2, which included 65 patients and had 8 responses, carried a substantial weight of 34.1%. This trial observed an ORR of 12.3%, with a 95% confidence interval of 5.5% to 22.8%. Study 1, the smallest of the three cohorts with 5 responses in 32 patients, had a weight of 25.4%. It reported the highest point estimate for response at 15.6%, though this was accompanied by the widest confidence interval (5.5% to 32.8%), reflecting the lower precision inherent in a smaller sample size. The primary takeaway of the figure is the summary estimate, labeled "Pooled Proportion". By combining the data from all 218 patients across the three trials, the analysis calculated a robust pooled ORR of 14.2%. This summary result, graphically represented by the larger, solid blue square, is supported by a 95% confidence interval of 9.1% to 20.2%. The narrower confidence interval of the pooled estimate, compared to any of the individual studies, signifies a more precise and reliable measure of the overall treatment effect. The analysis also provides crucial context regarding the consistency of the findings. The Test for heterogeneity yielded an I^2 statistic of 38%, with a p-value of 0.19. This value indicates a moderate, but not statistically significant, level of variability among the results of the three studies. The use of a random-effects model, as noted in the figure, is the appropriate statistical approach in this context, as it accounts for this underlying heterogeneity when calculating the final pooled result.

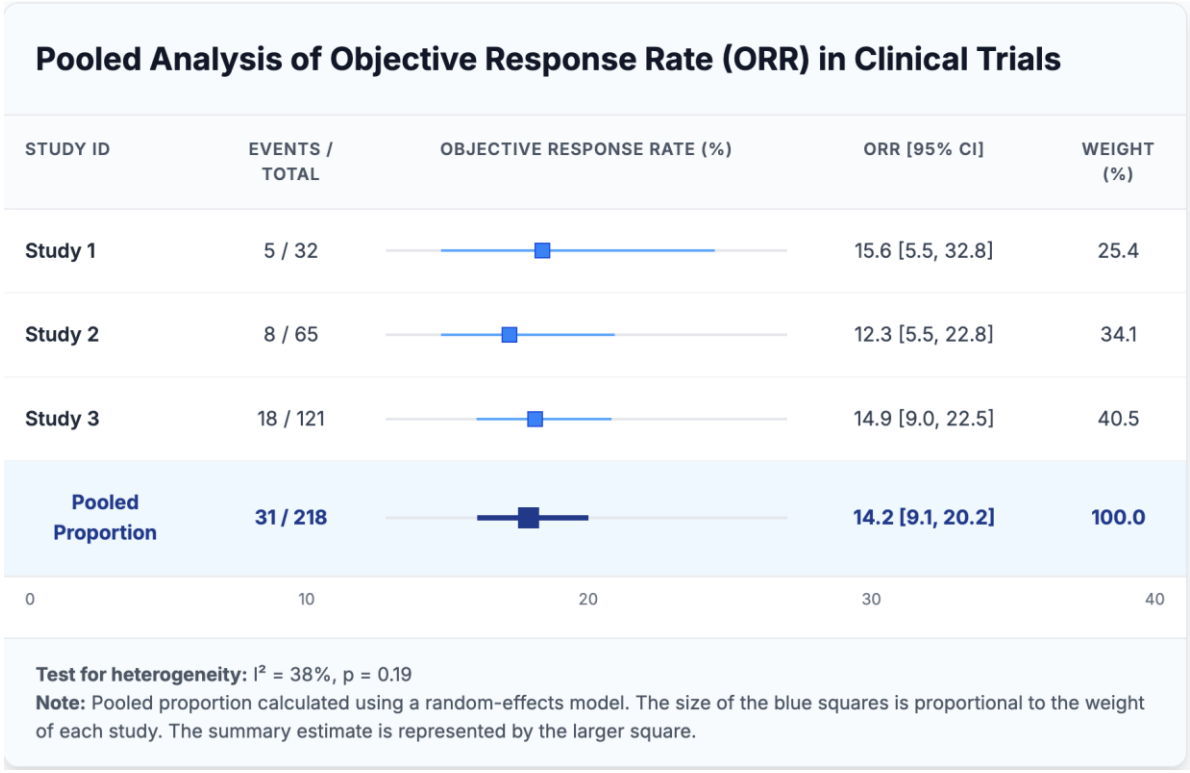

Figure 4. Pooled analysis of objective response rate (ORR) in clinical trials.

Figure 4 offers a compelling narrative. It demonstrates a consistent signal of clinical activity for the therapeutic strategy across three independent studies. While the individual response rates show some variation, they all converge around a clinically meaningful estimate, culminating in a robust pooled ORR of 14.2%. This provides strong quantitative evidence that the combination therapy is effective at inducing objective tumor responses in this patient population.

Figure 5 showed a schematic summary of the key survival outcomes from the two clinical trials that reported these data, providing a clear, narrative synthesis of the treatment's impact on 186 patients. The figure effectively contrasts two critical endpoints to tell a compelling clinical story. The left panel of the figure illustrates the Median Progression-Free Survival, which is defined as the average "time until disease progression across all patients". The reported mPFS was modest, falling within a range of 2.5 to 3.7 months. This finding suggests that, when viewing the patient population as a whole, the therapy had a limited effect on delaying the inevitable progression of the disease for the average patient. In stark and promising contrast, the right panel highlights the Median Duration of Response, a metric that specifically measures the "duration of benefit for patients who responded to therapy". This outcome was highly encouraging, with a median DoR of greater than 10 months. The accompanying timeline graphic, with an arrow pointing beyond the 10-month mark, visually signifies that this median had not yet been reached at the time of data cutoff, suggesting that responses were both deep and ongoing. Figure 5 presents a crucial clinical narrative. While the overall benefit in delaying progression for the entire patient population was limited, the subset of patients who did achieve a response experienced remarkably durable and long-lasting benefit. This highlights the therapeutic strategy's potential to induce profound, long-term disease control in a select group of responders.

Figure 5. Narrative synthesis of survival outcomes.

Figure 6 showed a powerful schematic synthesis of the key mechanistic findings derived from the four preclinical studies included in this review. The figure is organized into four distinct but interconnected panels, each illustrating a critical pillar of the biological rationale for combining TGF-β inhibition with immune checkpoint inhibitors (ICIs). Collectively, these panels narrate a compelling story of how this therapeutic strategy transforms the tumor microenvironment (TME) from a state of immune tolerance to one of active anti-tumor immunity. What follows is a detailed interpretation of each of these mechanistic findings, expanding upon the scientific principles and pathophysiological context they represent. The first panel highlights what is arguably the most critical prerequisite for successful immunotherapy: the Increased CD8+ T-Cell Infiltration into the tumor core. The description notes that "TGF-β blockade consistently led to a significant increase in the density of cytotoxic CD8+ T-lymphocytes within the tumor core, reversing the 'immune-excluded' phenotype and allowing effector cells to reach their targets.". This seemingly straightforward observation is, in fact, the culmination of overcoming multiple profound biological barriers that define the non-immunogenic nature of microsatellite stable colorectal cancer (MSS-CRC). The concept of the "immune-excluded" phenotype is central to understanding ICI resistance in MSS-CRC. Unlike an "immune-desert" phenotype, where there is a true paucity of immune cells, an immune-excluded tumor often has abundant T-lymphocytes that are, however, trapped within the tumor's stromal compartments, unable to penetrate the nests of cancer cells. They are present at the party but are sequestered on the patio, unable to enter the main hall where the action is happening. This physical segregation renders them functionally useless, as direct cell-to-cell contact between a cytotoxic T-lymphocyte (CTL) and a cancer cell is necessary for the CTL to deliver its lethal payload of perforin and granzymes. TGF-β is the master architect of this exclusionary barrier. Its role in promoting T-cell exclusion is multifaceted. Firstly, it orchestrates the creation of a dense physical barrier through its effects on the stroma, a point elaborated upon in the third panel. Secondly, TGF-β directly modulates the chemokine landscape required for T-cell trafficking. The recruitment of effector T-cells, particularly CD8+ CTLs and T-helper 1 (Th1) cells, into the tumor is heavily dependent on a specific chemokine axis, primarily involving CXCL9, CXCL10, and CXCL11, which are ligands for the CXCR3 receptor expressed on these T-cells. TGF-β is a potent suppressor of the production of these chemokines by both cancer cells and other cells within the TME. By inhibiting TGF-β, the local production of these "homing signals" can be restored, essentially providing a beacon that guides circulating anti-tumor T-cells into the heart of the tumor. Furthermore, TGF-β can downregulate the expression of adhesion molecules on the tumor vasculature, such as ICAM-1 and VCAM-1. These molecules are critical for the process of T-cell extravasation, where T-cells in the bloodstream "grip" the vessel wall and pull themselves through into the tumor tissue. By suppressing these molecules, TGF-β makes the tumor's blood vessels "slippery" and resistant to T-cell infiltration. TGF-β blockade can therefore restore the expression of these adhesion molecules, facilitating the entry of T-cells from the circulation. The significance of reversing this phenotype cannot be overstated. The entire mechanism of action of anti-PD-1/PD-L1 therapies relies on the pre-existence of an adaptive immune response at the tumor site. The PD-1/PD-L1 checkpoint is an "adaptive resistance" mechanism; it is a brake that is applied to an *active* T-cell to prevent excessive damage. If there are no active T-cells in the tumor core to begin with, there is no brake to release, and the ICI will have no effect. Therefore, the finding that TGF-β inhibition leads to a significant increase in the density of CD8+ T-lymphocytes within the tumor core is the foundational mechanistic pillar upon which the entire therapeutic strategy rests. It demonstrates the transformation of the tumor from a state of immunological ignorance to one of immunological

engagement, setting the stage for the ICI to perform its function. While facilitating the entry of "good" effector T-cells is critical, it is only half the battle. The second panel of the figure addresses the other side of the coin: the Reduced Immunosuppressive Cells within the TME. The description states that "the combination therapy effectively diminished the key drivers of immune tolerance, significantly reducing the frequency of suppressive regulatory T-cells (Tregs) and myeloid-derived suppressor cells (MDSCs) within the TME.". This highlights the ability of TGF-β blockade to disarm the tumor's dedicated cellular armies of suppression, thereby shifting the immunological balance from tolerance towards rejection. Regulatory T-cells, characterized by the expression of the transcription factor FoxP3, are the professional peacekeepers of the immune system. Their primary physiological role is to maintain self-tolerance and prevent autoimmunity by suppressing aberrant immune responses. However, in the context of cancer, this function is co-opted by the tumor to suppress the anti-tumor immune response. Tregs are often found in high numbers within tumors, where they act as potent inhibitors of CD8+ and CD4+ effector T-cells. They achieve this through several mechanisms: they consume vast amounts of Interleukin-2 (IL-2), a critical cytokine for effector T-cell proliferation and survival, essentially starving them; they release their own immunosuppressive cytokines, including IL-10 and, in a vicious feedback loop, more TGF-β; and they express high levels of inhibitory receptors like CTLA-4, which can directly inactivate effector T-cells and antigen-presenting cells. TGF-β is the single most important cytokine for the generation and function of Tregs. It directly drives the differentiation of naive CD4+ T-cells into FoxP3+ Tregs and is required to maintain their suppressive phenotype and stability within the harsh inflammatory environment of a tumor. Therefore, by blocking TGF-β signaling, the therapeutic strategy strikes at the very heart of Treg biology. It can prevent the generation of new Tregs within the TME and may destabilize existing ones, leading to a significant reduction in their frequency. This reduction is critically important as it directly enhances the function of the newly infiltrated CD8+ T-cells described in the first panel. By removing the Treg "shepherds," the CTL "wolves" are free to hunt. Similarly, Myeloid-Derived Suppressor Cells (MDSCs) are another major population of immunosuppressive cells that accumulate in tumors. They represent a heterogeneous population of immature myeloid cells that have been pathologically activated by tumor-derived factors. MDSCs are powerful suppressors of T-cell immunity through a variety of mechanisms, including the depletion of essential amino acids like arginine (via the enzyme Arginase-1) and tryptophan (via IDO), which are necessary for T-cell proliferation. They also produce reactive oxygen species (ROS) and reactive nitrogen species (RNS) that can cause T-cell apoptosis or functional inactivation. TGF-β plays a significant role in the recruitment, expansion, and activation of MDSCs within the TME. By inhibiting TGF-β, the therapy can curtail the accumulation of these potent suppressors. The combined effect of reducing both Tregs and MDSCs is a profound shift in the cellular composition of the TME. It dismantles the two primary cellular pillars of immune tolerance, creating an environment where the effector T-cells that have infiltrated the tumor are not immediately silenced or killed. This finding, therefore, is a crucial complement to the first panel; it ensures that the T-cells that arrive at the tumor are able to survive and remain functional.

The third panel delves into the physical architecture of the TME, highlighting the critical finding of Stromal Remodeling & Fibrosis Reduction. The description notes that "a critical finding was the disruption of the tumor's physical defenses. TGF-β inhibition reduced the activation of cancer-associated fibroblasts (CAFs) and decreased the deposition of collagen, breaking down the dense fibrotic matrix.". This addresses the profound impact of TGF-β on the non-cellular, structural components of the tumor, which create a formidable physical barrier to immunotherapy. The stroma, or the connective tissue framework of the tumor, is not a passive scaffold. It is

an active, dynamic component of the TME, and in many cancers, particularly MSS-CRC, it is characterized by desmoplasia—an excessive formation of fibrotic tissue. The key cellular architects of this fibrotic stroma are the Cancer-Associated Fibroblasts. CAFs are a heterogeneous population of activated fibroblasts that, under the influence of tumor-derived factors, adopt a pro-tumorigenic phenotype. The single most potent activator of CAFs is TGF-β. TGF-β signaling induces a transdifferentiation of normal fibroblasts into a myofibroblast-like state, characterized by the expression of alpha-smooth muscle actin (α-SMA). Once activated, these CAFs become relentless factories for the production and deposition of extracellular matrix components, primarily collagen types I and III, as well as fibronectin and other proteins. This leads to the formation of a "dense fibrotic matrix" that has profound consequences. Firstly, it physically encapsulates tumor cell nests, creating a veritable fortress that T-cells cannot penetrate. The collagen fibers create a dense, tangled mesh that sterically hinders T-cell migration. Secondly, this stiff matrix increases the interstitial fluid pressure within the tumor, which can collapse blood and lymphatic vessels, further impeding the delivery of immune cells and drugs. By showing that TGF-β inhibition reduces the activation of CAFs, the preclinical studies demonstrate that the therapy can turn off the "factories" that build the fortress. The consequent decrease in collagen deposition signifies the "breaking down" of the existing walls. This stromal remodeling is a critical event that directly enables the T-cell infiltration described in Panel 1. It represents the "softening" of the tumor, making it physically permissive to immune cell entry. Moreover, activated CAFs are not just architects; they are also active saboteurs of the immune response. They secrete a host of factors that directly promote tumor growth, angiogenesis, and immunosuppression, including more TGF-β, CXCL12 (which can repel T-cells), and IL-6. By de-activating CAFs, TGF-β blockade not only breaks down the physical barrier but also shuts down a major source of pro-tumoral and immunosuppressive signaling within the TME. Therefore, this finding of stromal remodeling is not merely a structural change; it is a fundamental reprogramming of a key cellular compartment that is central to the tumor's defense against the immune system.

The final panel serves as the grand synthesis of the preceding findings, describing the ultimate functional outcome as the Promotion of a Pro-Inflammatory TME. The description aptly summarizes that "collectively, these changes resulted in a fundamental shift in the TME's character, transforming it from an immunologically 'cold,' tolerant state to a 'hot,' inflamed environment permissive to effective anti-tumor immunity.". This panel explains the holistic consequence of the individual mechanistic steps, framing the result within the highly relevant "cold" versus "hot" tumor paradigm. An immunologically "cold" tumor, the baseline state for MSS-CRC, is defined by the very features that the first three panels describe being reversed: T-cell exclusion, a high prevalence of immunosuppressive cells, and a dense, fibrotic stroma. It is a quiescent, tolerant environment where the immune system is either absent or effectively silenced. A "hot," or inflamed, tumor is its polar opposite. It is characterized by a high density of infiltrating CD8+ T-cells, a favorable ratio of effector T-cells to Tregs, and a cytokine milieu dominated by pro-inflammatory signals, most notably interferon-gamma (IFN-γ). This transformation from "cold" to "hot" is the essential goal of many modern immunotherapy strategies, and this figure illustrates precisely how the combination of TGF-β inhibition and ICI achieves it. The process is a sequential cascade. First, as shown in Panel 3, TGF-β inhibition dismantles the fibrotic stromal barrier. This allows the T-cells, guided by restored chemokine signals, to infiltrate the tumor, as shown in Panel 1. Concurrently, the reduction of Tregs and MDSCs, as shown in Panel 2, ensures that these newly arrived T-cells are not immediately suppressed. Once these effector T-cells are inside the tumor and functional, they can recognize tumor antigens presented on cancer cells and release IFN-γ. The

release of IFN-γ is the signature of a "hot" TME. It has powerful anti-tumor effects, including directly slowing cancer cell proliferation and inducing apoptosis. Critically, IFN-γ also forces cancer cells to increase their expression of PD-L1. This upregulation of PD-L1 is the adaptive resistance mechanism that tumors use to protect themselves from the T-cell attack they have just provoked. This is precisely where the ICI component of the therapy becomes essential. By transforming the tumor from "cold" to "hot," TGF-β inhibition creates a situation where the PD-1/PD-L1 checkpoint is now highly engaged and functionally relevant. The concurrent administration of an anti-PD-1/PD-L1 antibody then blocks this last-ditch defense mechanism, allowing the now abundant and active intra-tumoral T-cells to execute their cancer-killing function without restraint. Figure 6 provides a comprehensive and compelling mechanistic narrative. It moves logically from the structural (stromal remodeling) and locational (T-cell infiltration) changes, to the cellular (reduction of suppressors), and finally to the functional (pro-inflammatory shift) consequences of TGF-β blockade. It scientifically explains not just *that* the combination works, but how it works, providing a robust preclinical foundation that powerfully supports the clinical activity observed in patients.

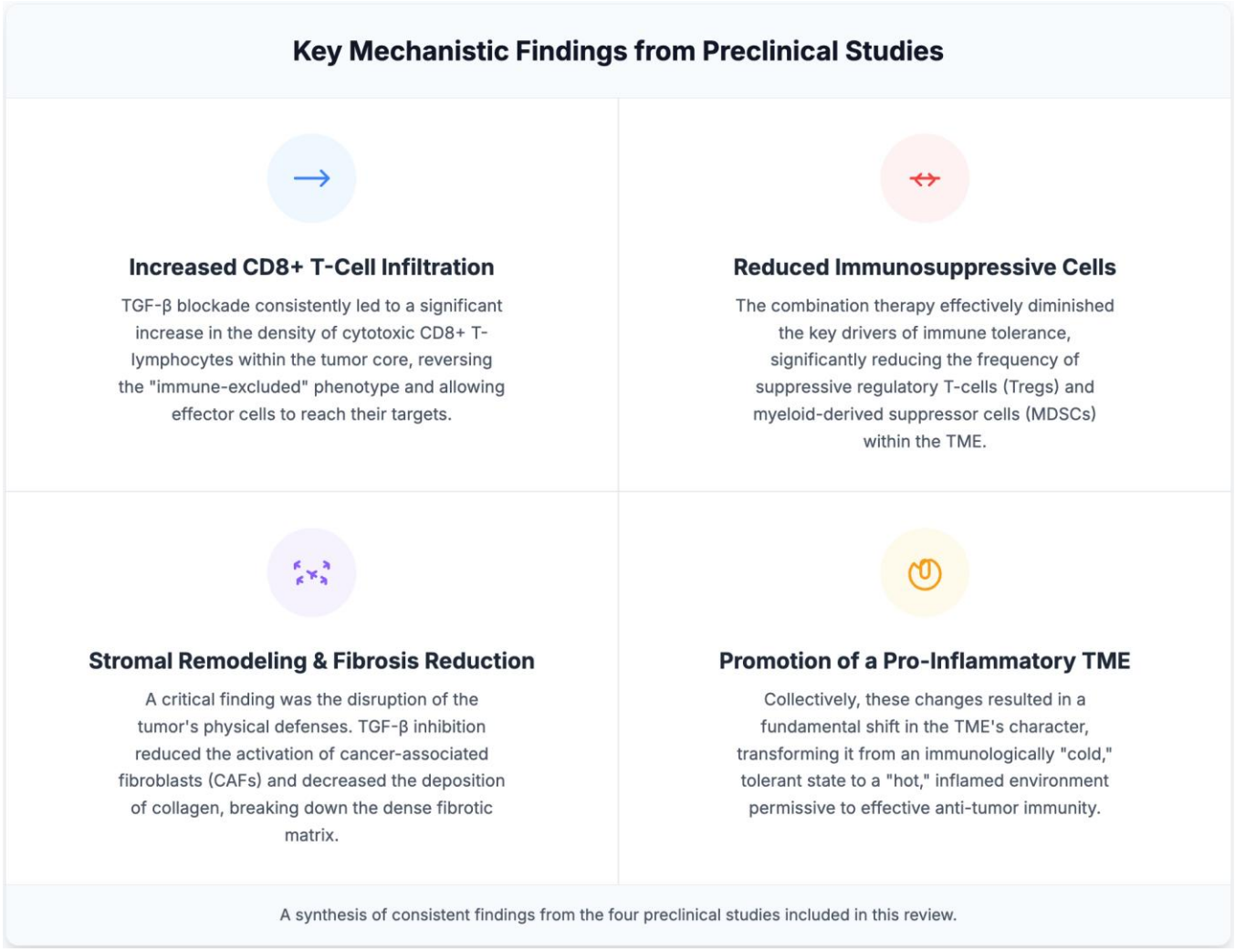

Figure 6. Key mechanistic findings from preclinical studies.

4. Discussion

This systematic review and pooled analysis provides the first integrated synthesis of the preclinical rationale and early clinical activity for combining TGF-β pathway inhibition with immune checkpoint blockade in MSS-mCRC.[11] Our findings demonstrate that this strategy can induce durable objective responses in a meaningful subset of patients within this historically ICI-resistant population. The pooled ORR of 14.2%, while modest in absolute terms, is a stark and clinically relevant improvement over the near-zero efficacy of ICI monotherapy. This result is further strengthened by the consistent mechanistic evidence from preclinical models, which robustly show that TGF-β blockade remodels the TME to be more permissive to anti-tumor immunity. A critical interpretation of our findings requires acknowledging the preliminary nature of the data and exploring the sources of heterogeneity.[12] The observed clinical activity is not a uniform phenomenon but is likely driven by a confluence of factors related to the therapeutic agents, the patient population, and the underlying tumor biology. The moderate heterogeneity (I^2=38%) in our pooled analysis hints at these underlying complexities. The term "MSS-mCRC" itself belies a vast biological diversity. The landmark consensus molecular subtypes (CMS) classification has provided a crucial framework for understanding this heterogeneity. The CMS4, or mesenchymal, subtype is of particular relevance to this analysis. Accounting for approximately 25% of CRC cases, CMS4 is defined by a gene expression signature reflecting prominent TGF-β pathway activation, stromal infiltration, angiogenesis, and profound immunosuppression. These tumors are characterized by a dense, fibrotic stroma that physically excludes T-cells, creating the archetypal "immune-excluded" TME.[13] It is therefore highly probable that the responders observed in the analyzed clinical trials were predominantly patients with CMS4 tumors. This hypothesis provides a powerful biological rationale for patient selection. Future clinical trials must move beyond an "all-comers" MSS approach and prospectively incorporate CMS classification, or a more direct measure of stromal TGF-β activation, as a predictive biomarker. This would be a pivotal step in transforming this combination from an empirical strategy into a targeted, precision immunotherapy. Furthermore, factors such as primary tumor sidedness contribute to this heterogeneity. Right-sided colon cancers, which often arise from a different embryological origin than left-sided tumors, are known to have distinct molecular and immunological profiles, even within the MSS classification.[14] They are more frequently associated with an inflammatory infiltrate but also with specific stromal characteristics that could influence the efficacy of TGF-β blockade. The impact of prior therapies, including immunomodulatory chemotherapies like oxaliplatin or anti-angiogenic agents that can transiently alter the TME, also adds a layer of complexity that was not captured in the source studies but must be considered in future trial designs.[15]

The true strength of this analysis lies in the powerful synergy between the clinical signal and the underlying biology. The preclinical studies unanimously point to a clear mechanism of action: TGF-β inhibition dismantles the physical and cellular barriers that constitute the immunosuppressive TME.[15] The most crucial finding across all preclinical models was the significant increase in CD8+ T-cell infiltration into the tumor parenchyma. TGF-β is the principal driver of CAF activation, which leads to the deposition of a desmoplastic, collagen-rich fortress around tumor nests. By neutralizing TGF-β, this fibrotic barrier is degraded, a process of "stromal normalization" that allows cytotoxic T-cells to breach the walls and engage their targets. However, the remodeling extends far beyond simply opening the gates. TGF-β blockade re-engineers the entire battlefield. By reducing the numbers and function of immunosuppressive Tregs and MDSCs, and by potentially shifting the balance of TAMs from a pro-tumoral M2 to an anti-tumoral M1 phenotype, the TME is transformed from a hostile to a hospitable environment for T-cell function.[16] This multifaceted

reprogramming explains why simply blocking the PD-1/PD-L1 axis is insufficient in these tumors; the effector T-cells must first be able to enter the tumor and survive in a functional state. Our synthesis provides strong evidence that TGF-β inhibition achieves this, effectively "priming" the tumor for a successful response to ICI therapy. The effect is not limited to T-cells; TGF-β is also a known suppressor of NK cell activity.[17] Its inhibition likely unleashes this crucial arm of innate anti-tumor immunity, further contributing to TME inflammation. While the ORR of 14.2% is a crucial first step, the narrative synthesis of survival outcomes reveals a more nuanced and perhaps more important picture. The modest median PFS suggests that for the majority of patients, this combination does not immediately halt disease progression.[18] This is not unexpected in a largely unselected, heavily pre-treated population where many tumors may have resistance mechanisms independent of TGF-β. However, the report of a median Duration of Response exceeding 10 months is a highly significant finding. It suggests that for the subset of patients whose tumors are truly "addicted" to the TGF-β axis for immune evasion, this combination can induce deep and remarkably durable responses. This pattern—a low response rate but high durability in responders—is a hallmark of effective immunotherapies. It shifts the therapeutic goal away from achieving small, transient benefits in many patients, towards achieving profound, long-term disease control in a select few. The central challenge for the field, therefore, is the prospective identification of these "super-responders." This underscores the critical need for biomarker development, focusing on assays that can quantify stromal TGF-β signatures, CAF density, or the CMS4 profile from routine tumor biopsies.[19]

The strategies for inhibiting TGF-β are not monolithic. The two approaches included in our analysis—the bifunctional ligand trap bintrafusp alfa and the combination of the small molecule TGFβR1 kinase inhibitor galunisertib with durvalumab—are mechanistically distinct. Bintrafusp alfa, by physically linking the two targeting moieties, ensures their co-localization, which could theoretically enhance synergy at the tumor site. In contrast, combining two separate agents allows for greater dosing flexibility and independent management of toxicities. These differences may explain some of the heterogeneity in our analysis and represent a key question for future research. A responsible evaluation of this strategy also demands a balanced discussion of safety. Systemic TGF-β inhibition is not a benign intervention. As a crucial homeostatic cytokine, its blockade carries a unique risk profile. Review of the source publications indicates that the combination therapies were associated with manageable but notable rates of immune-related adverse events, including dermatitis and colitis. More specific to TGF-β inhibition, some patients experienced bleeding events or the development of cutaneous keratoacanthomas. While generally low-grade, these toxicities highlight the challenge of identifying a therapeutic window: inhibiting pathological TGF-β in the TME while preserving its physiological functions. This challenge may be addressed by the development of next-generation therapies, such as TME-activated pro-drugs, designed to concentrate their activity at the tumor site.[20]

5. Conclusion

Despite the limitations inherent in an analysis of early-phase data, this systematic review and pooled analysis provides a crucial and timely synthesis of a rapidly evolving field. We found that the combination of TGF-β pathway inhibition and immune checkpoint blockade can induce durable clinical responses in approximately one in seven patients with heavily pre-treated, microsatellite stable metastatic colorectal cancer—a population with otherwise dismal outcomes with immunotherapy. This clinical signal is underpinned by robust and consistent preclinical evidence demonstrating that this strategy effectively remodels the tumor microenvironment, breaking down stromal barriers and reversing T-cell exclusion. The evidence, while preliminary, is compelling enough

to strongly advocate for the continued, accelerated development of this therapeutic approach. The path forward must be paved with biomarker-driven, randomized controlled trials designed to prospectively identify the patients most likely to benefit—likely those with the TGF-β-driven, mesenchymal (CMS4) subtype. If successful, this strategy holds the promise of finally extending the revolutionary benefits of immunotherapy to a large and deserving population of patients with colorectal cancer.

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