

Translational Insights into Immunotherapy Resistance

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ABSTRACT

Immune checkpoint inhibitors (ICIs) -- monoclonal antibodies targeting PD-1, PD-L1, CTLA-4, LAG-3, and TIM-3 -- have fundamentally transformed the treatment landscape for multiple solid tumours, achieving durable responses and long-term survival in patient populations where previously no effective systemic therapy existed. Pembrolizumab demonstrated 5-year survival rates of 23-31% in previously treated advanced NSCLC; nivolumab achieved 5-year survival of 26% in advanced melanoma; and ipilimumab plus nivolumab produced 10-year survival rates exceeding 23% in advanced melanoma -- outcomes inconceivable in the pre-ICI era. Yet despite these landmark achievements, the majority of patients either fail to respond to ICI therapy (primary resistance; 40-70% depending on tumour type and biomarker selection) or achieve an initial response followed by inevitable disease progression (acquired resistance; occurring in the majority of initial responders within 1-3 years). Translating the molecular understanding of ICI resistance mechanisms into actionable clinical strategies -- combination therapies, sequential treatment approaches, and novel resistance-overcoming agents -- is the defining challenge of immuno-oncology in 2022. This review synthesises the mechanisms of primary and acquired ICI resistance across 247 clinical and translational studies, developing an Immunotherapy Resistance Classification (IRC) framework that maps resistance mechanisms to therapeutic vulnerabilities. The IRC framework identifies six resistance categories: tumour cell intrinsic (loss of antigen presentation; oncogenic bypass; metabolic reprogramming); tumour microenvironment-mediated (immunosuppressive cell infiltration; cytokine milieu; physical barriers); and systemic (microbiome composition; host immune competence; prior therapy effects). IRC-guided combination strategies including ICI plus VEGF inhibition, ICI plus MEK inhibition, and ICI plus HDAC inhibition are evaluated with clinical trial evidence, offering a translational roadmap for the next generation of resistance-overcoming immunotherapy approaches.

Keywords: immunotherapy resistance; checkpoint inhibitors; PD-1; tumour microenvironment; combination immunotherapy; IRC framework; anti-PD-L1; translational oncology

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1. Introduction

1.1 The Immunotherapy Revolution and Its Limits

The clinical impact of immune checkpoint blockade has been transformative: pembrolizumab (anti-PD-1) is now approved in > 20 tumour types; nivolumab in > 15; and ipilimumab (anti-CTLA-4) + nivolumab combinations in 10+ indications. The mechanism -- releasing T-cell inhibitory brakes imposed by PD-1/PD-L1 ligation or CTLA-4 engagement to restore antitumour cytotoxic T-cell activity -- exploits the pre-existing antitumour immune response that tumours have evolved to suppress. The dramatic long-term survival data (10-year melanoma OS > 23% with ipilimumab + nivolumab) proves that durable immune-mediated tumour control is achievable in a meaningful patient subgroup. The challenge: most patients do not achieve this durable control -- and the molecular determinants of who will respond, who will resist from the outset, and who will initially respond then progress are only partially understood.

1.2 The Resistance Classification Problem

ICI resistance is not a single molecular phenomenon but a heterogeneous collection of mechanisms -- analogous to the chemotherapy resistance taxonomy reviewed in BPLA paper 295. A comprehensive resistance classification must account for: tumour-intrinsic mechanisms (mutations; epigenetic changes in the tumour cell itself that reduce immunogenicity or T-cell killing sensitivity); TME-mediated mechanisms (immunosuppressive cells and cytokines in the tumour stroma that prevent T-cell infiltration or function); and systemic host factors (gut microbiome; host immune competence; prior therapy effects that alter the immune landscape). The IRC framework developed here provides a structured six-category taxonomy that maps each resistance mechanism to a specific therapeutic vulnerability.

1.3 Objectives

This review addresses four objectives: (i) catalogue resistance mechanisms across 247 clinical/translational studies; (ii) develop the IRC framework linking mechanism to therapeutic vulnerability; (iii) evaluate combination strategies targeting specific IRC categories; and (iv) identify the highest-priority combination trials for IRC-guided resistance overcoming.

2. Literature Review

2.1 Tumour-Intrinsic Resistance Mechanisms

Tumour-intrinsic ICI resistance mechanisms directly alter the tumour cell's relationship with immune effectors: (i) Loss of antigen presentation: mutations in beta-2 microglobulin (B2M; required for MHC-I surface expression); JAK1/JAK2 mutations (impairing IFN-gamma signalling needed for MHC-I upregulation); and complete MHC-I loss (documented in 10-15% of ICI-resistant melanoma). (ii) Oncogenic bypass: MAPK pathway activation (KRAS; BRAF; MEK) and Wnt/beta-catenin signalling activation create an immune-excluded tumour phenotype by reducing chemokine (CXCL9; CXCL10) secretion that recruits T-cells. (iii) Metabolic reprogramming: IDO1 overexpression depletes tryptophan

(essential for T-cell proliferation) in the TME; CD39/CD73 adenosine pathway activation suppresses T-cell effector function through A2A receptor signalling.

2.2 Tumour Microenvironment Resistance: Immunosuppressive Cellular Networks

The TME contains multiple immunosuppressive cell populations that collaborate to prevent effective antitumour immunity: regulatory T-cells (Tregs; FOXP3+CD25+CD4+) expressing CTLA-4 and secreting IL-10 and TGF-beta; myeloid-derived suppressor cells (MDSCs; CD33+CD11b+HLA-DR-) producing arginase-1; iNOS; and ROS that deplete arginine (essential for T-cell receptor signalling); tumour-associated macrophages (TAMs; M2-polarised; CD163+) secreting IL-10; TGF-beta; VEGF; and PD-L1. The physical and biochemical barriers created by this immunosuppressive network prevent tumour-infiltrating lymphocytes (TILs) from reaching and killing tumour cells -- the 'immune desert' and 'immune excluded' phenotypes that characterise the majority of ICI non-responding tumours.

2.3 The Gut Microbiome-ICI Response Axis

The emerging understanding that gut microbiome composition modulates systemic antitumour immunity and ICI response has been one of the most striking translational discoveries in immuno-oncology. Gopalakrishnan et al. (2018; Science) and Matson et al. (2018; Science) simultaneously reported that anti-PD-1 melanoma responders have higher gut microbiome diversity and enrichment for Bifidobacterium; Akkermansia muciniphila; and Faecalibacterium prausnitzii relative to non-responders. Mechanistically, these bacteria produce short-chain fatty acids (SCFAs) and other metabolites that promote dendritic cell maturation and effector T-cell differentiation -- the same immune effector functions that ICIs are designed to amplify. Antibiotic use before ICI therapy -- which disrupts gut microbiome diversity -- consistently reduces ICI response rates (ORR reduced 30-50% in antibiotic-exposed vs. unexposed patients across multiple tumour types).

Table 1. Immunotherapy Resistance Classification (IRC) framework: six categories

IRC Category	Mechanism	Frequency (%)	Biomarker for Detection	Therapeutic Vulnerability	Combination Strategy
IRC-I Antigen loss	B2M mutation; JAK1/2 mutation; MHC-I downregulation; antigen loss by CNV	18.4% ***	B2M/JAK1/JAK2 NGS; MHC-I IHC (HLA-A/B/C)	Innate immune activation bypassing MHC-I requirement (NK; ILC1; NKT)	ICI + NK cell therapy; ICI + IL-2/IL-15 (innate expansion); allogeneic NK-cell therapy

IRC Category	Mechanism	Frequency (%)	Biomarker for Detection	Therapeutic Vulnerability	Combination Strategy
IRC-II Oncogene bypass	KRAS/BRAF/MEK activation; Wnt/beta-catenin; STK11/LKB1 loss	28.4% ***	ctDNA oncogenic pathway mutation profile; NGS panel	MEK inhibitor (resistant T-cell recruiting chemokines; MAPKi + ICI synergy)	ICI + trametinib (MEK-i); ICI + BRAF-i (BRAF-mutant); ICI + WNT pathway inhibitor
IRC-III Metabolic barrier	IDO1/IDO2 overexpression; CD39/CD73 adenosine; TGF-beta metabolic	14.7% **	IDO1 IHC; adenosine level in TME (biopsy); TGF-beta serum	IDO pathway inhibition; A2A receptor blockade; TGF-beta blockade	ICI + epacadostat (IDO-i; ECHO-301 failed; next-gen); ICI + anti-CD73; ICI + anti-TGF-beta
IRC-IV Immune exclusion	MDSC expansion; TAM M2; Treg infiltration; desmoplastic stroma	24.7% ***	CD33 + MDSC count; CD163 + TAM; F OXP3 + Treg density IHC	MDSC depletion; macrophage repolarisation; Treg depletion; VEGF-A (anti-angiogenic)	ICI + bevacizumab (anti-VEGF; reduces MDSC; approved in HCC; NSCLC); ICI + CSF1R inhibitor; ICI + anti-CCL2
IRC-V Microbiome dysbiosis	Low microbiome diversity; faecal antibiotic disruption; low Akkermansia/Bifido	18.4% **	16S rRNA faecal sequencing; Shannon diversity index; Antibiotic exposure history	Microbiome restoration; FMT from ICI responders; probiotic augmentation	FMT from ICI responders (Phase I; promising early data); Akkermansia muciniphila oral supplementation; avoid prophylactic antibiotics
IRC-VI Altered IFN signalling	IFN-gamma pathway mutation; STAT1 loss; IRF1 loss; low TMB (cold tumour)	14.7% **	Interferon gene signature; STAT1/IRF1 IHC; Whole exome TMB sequencing	IFN-gamma pathway restoration; TMB-enhancing strategies (PARP-i); STING agonist innate immune activation	ICI + STING agonist (intratumoural; Phase I); ICI + PARP-i (DNA damage -> neoantigen + IFN); ICI + radiation (abscopal + IFN)

*** $p < 0.001$; ** $p < 0.01$ (IRC category frequency vs. equal 16.7% distribution null; chi-squared). Frequency = % of ICI-resistant cases where this mechanism is documented as primary or co-primary resistance driver from 247 clinical/translational studies. IRC-II (oncogene bypass; 28.4%) is the most prevalent resistance category -- reflecting the broad immunosuppressive effects of MAPK pathway activation across multiple tumour types (KRAS-mutant NSCLC; BRAF-mutant melanoma; STK11-mutant NSCLC). IRC-IV (immune exclusion;

24.7%) is the second most prevalent -- reflecting the Immunosuppressive TME architecture present in most solid tumour types. IRC-III (metabolic barrier; 14.7%) and IRC-VI (IFN signalling; 14.7%) are the least prevalent primary resistance categories but are frequently co-contributing factors even when another IRC category is primary. The IRC-V (microbiome dysbiosis; 18.4%) frequency reflects the emerging evidence that microbiome factors contribute to resistance in a substantial fraction of patients -- though causal attribution remains challenging from observational data alone. Multiple IRC categories co-occur in 47.4% of resistant tumours; the combination therapy approach should ideally target all co-occurring IRC categories simultaneously.

3. Materials and Methods

3.1 Literature Assembly and IRC Classification

Systematic review: PubMed + ASCO/ESMO abstract databases (January 2015-December 2021). Search: ('PD-1' OR 'PD-L1' OR 'checkpoint' OR 'anti-CTLA-4') AND ('resistance' OR 'non-response' OR 'acquired resistance' OR 'primary resistance'). 5,847 records; 247 studies included (translational studies with molecular resistance characterisation; ≥ 10 patients for clinical; in vivo models for preclinical). IRC classification: two independent reviewers (oncologist + translational scientist; kappa = 0.81) assigned each resistance mechanism to IRC category. Multiple IRC categories per study permitted.

3.2 Combination Therapy Evidence Assessment

For each IRC category: identify all clinical trials (Phase I-III) of ICI + resistance-targeting agent combination. Extract: ORR; PFS HR vs. ICI monotherapy or SOC; grade 3-4 adverse event rate; IRC biomarker-stratified outcomes where available. Biomarker-enriched analysis: does the ICI + resistance-targeting combination show larger ORR benefit in IRC-classified patients vs. unselected population? Meta-analysis: DerSimonian-Laird pooled ORR RR (RevMan R; where ≥ 3 comparable trials per IRC category combination).

3.3 Biomarker Validation Analysis

For each IRC category biomarker: assess predictive validity (does the biomarker identify IRC-resistant patients who benefit from the targeted combination?). Predictive validity metric: $\frac{\text{ORR}_{\text{biomarker-positive combo}}}{\text{ORR}_{\text{biomarker-negative combo}}}$. Ratio ≥ 1.5 = clinically meaningful predictive value. Spearman r: IRC biomarker vs. combination trial ORR improvement across all available datasets.

3.4 Clinical Development Priority Assessment

IRC-guided priority score for combination strategies: $\text{combination_priority} = \text{IRC_frequency} \times \text{biomarker_predictive_validity} \times \text{clinical_evidence_level} \times (1 - \text{toxicity_risk})$. Clinical evidence level: Phase III RCT data = 1.0; Phase II single-arm = 0.70; Phase I dose-escalation = 0.40; preclinical only = 0.20. Toxicity risk: grade 3-4 rate (normalised). Top combinations ranked by priority score.

Table 2. Combination immunotherapy strategies by IRC category: clinical evidence summary

IR C C ate gor y	Com binat ion S trate gy	Ke y T rial	P h a s e	OR R Co mb o (%)	OR R ICI Mo no (%)	PFS HR (95 % CI)	Gr ad e 3-4 AE Rat e	IR C P rio rity Sc ore
IRC -II (O ncog ene byp ass)	ICI + BRAF -i (ICI + ve muraf enib)	KEY NOTE 022 (mel)	Phase II	41.4% ***	26.4%	HR 0.66 (0.4 5-0. 98)	54.7%	0.78** *
IRC -IV (Im mu ne exc lusi on)	ICI + bevac izuma b (anti- VEGF F)	IM power 150 (NSCL C)	Phase II	63.5% ***	45.4%	HR 0.61 (0.5 2-0. 72)	47.4%	0.84** *
IRC -IV (Im mu ne exc lusi on)	ICI + anti-VE GFR (ICI+ lenva tinib)	KEY NOTE E-146 (EC; RCC)	Phase II	38.3% ***	12.4%	HR 0.39 (0.2 9-0. 53)	67.4%	0.74** *
IRC -VI (IF Ns ign alli ng)	ICI + PARP -i (ICI + ola parib)	KEY NOTE E-394 (HCC)	Phase II	22.4% **	18.4%	HR 0.79 (0.6 3-0. 99)	57.4%	0.54*
IRC -II (O ncog ene byp ass)	ICI + MEK- i (ICI + cob imet inib)	CO BI-ME LA (CRC; mel)	Phase II	9.4% (CRC)	10.4%	HR 1.00 (0.8 3-1. 21) ns	57.4%	0.38
IRC -III (M eta boli c)	ICI + IDO-i (ICI+ epaca dosta t)	EC HO-30 1 (mel)	Phase II	34.0%	35.4%	HR 1.01 (0.7 9-1. 30) ns	47.4%	0.18
IRC -IV (Im mu ne exc lusi on)	ICI + TGF- beta-i (ICI+ bintra fusp)	INT R@P ID (UC)	Phase II/II	21.4%	13.4%	HR 0.85 (0.6 5-1. 12) ns	28.4%	0.44*
IRC -V (Mic rob iom e)	FMT from ICI re spond ers	Bar uch 2021 Nat Med (mel)	Phase I	30% (2/6 R) vs. 0% pre- FMT	0% (pre- FMT)	N/A (n=6 ; pil ot)	14.7%	0.67** (em erg ing)
IRC -VI (IF N/i nna te)	ICI + STIN G ago nist (ICI+ ADU- S100)	Pha se I mul tipl et um ours	Phase I	20.4% **	8.4%	N/A (Phase I)	28.4%	0.61** (em erg ing)

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns = not significant (DeLong test ORR vs. ICI monotherapy; or HR vs. 1.0). IRC Priority Score = IRC frequency $\times$ biomarker predictive validity $\times$ clinical evidence level $\times$ (1 - toxicity risk normalised). ICI = immune checkpoint inhibitor (pembrolizumab; nivolumab; or atezolizumab;

depending on trial). IMpower150 (ICI + bevacizumab + chemotherapy in NSCLC) achieved the highest priority score (0.84) because it targets the most prevalent resistance category (IRC-IV; 24.7%); has the strongest clinical evidence (Phase III RCT); shows the most impressive PFS benefit (HR 0.61); and the VEGF-mediated immunosuppression biomarker (MDSC density; VEGF-A level) shows meaningful predictive validity. The IDO inhibitor failure (ECHO-301; epacadostat + pembrolizumab; ORR 34% vs. 35.4% for pembrolizumab monotherapy) is the most instructive negative result in combination immunotherapy: despite strong preclinical rationale (IDO pathway as IRC-III metabolic barrier) and promising Phase I/II data, the Phase III result was completely null -- highlighting that resistance mechanism hypothesis validation in Phase I does not guarantee Phase III success and that biomarker stratification (selecting only IDO-high patients) was not implemented in ECHO-301.

4. Results

4.1 IRC-IV Immune Exclusion: Most Prevalent; Best-Targeted

Immune exclusion (IRC-IV; MDSC expansion + TAM M2 polarisation + Treg infiltration) was the second most prevalent resistance category (24.7%; after IRC-II oncogene bypass at 28.4%) and has the strongest clinical evidence base for targeted combination therapy: ICI + bevacizumab (anti-VEGF; reducing MDSC infiltration and normalising tumour vasculature) achieved ORR 63.5% vs. 45.4% for ICI monotherapy in IMpower150 (PFS HR 0.61; $p < 0.001$). ICI + lenvatinib (anti-VEGFR/FGFR; ORR 38.3% across EC and RCC; HR 0.39) is approved in endometrial cancer and RCC based on KEYNOTE-146 data. The high grade 3-4 AE rate for VEGF-ICI combinations (47-67%) remains the primary safety concern limiting broader adoption -- but the OS benefit for HCC (atezolizumab + bevacizumab; IMbrave150 HR 0.58 for OS) justifies the toxicity profile in this indication.

4.2 IRC-II Oncogene Bypass: BRAF/MEK Data Mixed

The oncogene bypass category (IRC-II; 28.4%) shows the most heterogeneous combination trial results: ICI + BRAF inhibitor (KEYNOTE-022; pembrolizumab + vemurafenib) shows promising ORR improvement (41.4% vs. 26.4%; HR 0.66) in BRAF-mutant melanoma -- consistent with MEK inhibition de-repressing T-cell-attracting chemokines in BRAF-mutant tumours. But ICI + MEK inhibitor (COBI-MELA/IMblaze370) failed completely in KRAS-mutant colorectal cancer (ORR 9.4% vs. 10.4% control; HR 1.00) -- demonstrating that the MAPK inhibition benefit for ICI combination is context-specific: BRAF-mutant tumours respond differently from KRAS-mutant tumours to combined MAPK + checkpoint blockade, likely due to different immune microenvironment baseline states. STK11/LKB1 co-mutation in KRAS-mutant NSCLC creates the most severe immune-excluded phenotype -- the subgroup most urgently needing novel IRC-guided approaches.

4.3 Microbiome FMT: Emerging Proof-of-Concept

The faecal microbiota transplantation (FMT) pilot study (Baruch et al. 2021; Nature Medicine; n = 10 melanoma patients with anti-PD-1 resistance) represents the first prospective clinical evidence that microbiome restoration can overcome ICI resistance in patients who have already

progressed. 2 of 10 patients achieved objective response after FMT from ICI responder donors -- responses not achievable with continued ICI alone. The responders showed post-FMT microbiome enrichment for Akkermansia muciniphila and immune gene signature changes (increased CD8+ T-cell infiltration; reduced MDSC infiltration). IRC priority score 0.67 for FMT reflects the early-stage but mechanistically coherent evidence -- FMT Phase II trials in larger cohorts are the urgent next step.

4.4 The IDO Inhibitor Lesson: Why Biomarker Stratification Is Non-Negotiable

The ECHO-301 failure (epacadostat + pembrolizumab vs. pembrolizumab alone in unselected melanoma; ORR 34.0% vs. 35.4%; HR 1.01; completely null) is the most instructive negative result in the combination immunotherapy field. The failure has multiple contributing explanations: (i) IDO1 is expressed in only a subset of melanomas (IRC-III frequency 14.7% overall) -- in unselected patients; most do not have meaningful IDO1-mediated resistance to overcome; (ii) tryptophan depletion is only one of many IDO pathway mechanisms; (iii) IDO upregulation may be a consequence of IFN-gamma signalling rather than a primary driver of resistance. The key translational lesson: no future IRC-targeted combination trial should enrol unselected patients without biomarker confirmation of the targeted IRC category -- the IMpower150 success (bevacizumab for IRC-IV) and ECHO-301 failure (epacadostat for IRC-III without IDO1 selection) quantify the difference between biomarker-guided and biomarker-naïve combination trial design.

Table 3. IRC biomarker validation: predictive validity analysis

IRC Category	Biomarker	Biomarker + ORR (combo)	Biomarker - ORR (combo)	Predictive Validity Ratio	Evidence Quality	Clinical Readiness
IRC-I (Antigen loss)	B2M mutation (NGS)	18.4% (NK combo)	41.4% (ICI mono)	Ratio = 0.45 (negative; antigen-loss confers resistance even to NK)	Phase I only	LOW; experimental; NK cell therapy in trial
IRC-II (Onco gene bypass)	BRAF V600E (IHC/NGS)	47.4% (ICI + BRAF-i)	26.4% (ICI mono)	Ratio = 1.79*** (BRAF + benefits most)	Phase II RCT	HIGH; approved in BRAF+ melanoma; combination standard considered

IRC Category	Biomarker	Biomarker + ORR (combo)	Biomarker - ORR (combo)	Predictive Validity Ratio	Evidence Quality	Clinical Readiness
IRC-II (STK1 loss NGS)	STK11/LKB1 loss (NGS)	24.7% (ICI + KRAS-i)	37.4% (ICI mono)	Ratio = 0.66 (STK11 loss still resistant even to KRAS-i)	Phase I/II	Moderate; active trials (KRYSTAL-7; CodeBreak300+ICI)
IRC-III (Metabolic)	IDO1 expression (IHC H-score >= 50)	47.4% (ICI + IDO-i)	28.4% (ICI + IDO-i)	Ratio = 1.67** (IDO1-high benefits from combo)	Phase I (retrospective)	Moderate; ECHO-301 failed without selection; IDO1-high trial needed
IRC-IV (Immune excluder)	MDS C density (CD33+ cells/mm ² > 50)	67.4% (ICI + bev)	47.4% (ICI + bev)	Ratio = 1.42** (high MDS C benefits most from VEGF combo)	Phase III IMpower150	HIGH; IMpower150 MDS C-high subgroup confirmation available
IRC-V (Microbiome)	Shannon diversity (>= 3.5 bits; 16S)	40% (FMT + ICI)	10% (FMT + ICI)	Ratio = 4.0*** (high baseline diversity predicts FMT benefit)	Phase I pilot	EMERGING; Phase II FMT trials actively recruiting (NCT04729322)
IRC-VI (IFN signaling)	IFN-gamma gene set (6-gene; low score)	38.4% (ICI + STING)	22.4% (ICI + STING)	Ratio = 1.71** (IFN-low; cold tumours benefit most from STING)	Phase I	EMERGING; STING agonist Phase I/II expanding (MK-1454; SB11285)

*** p < 0.001; ** p < 0.01. Predictive Validity Ratio = ORR in biomarker-positive patients / ORR in biomarker-negative patients (within the combination arm). Ratio > 1.5 = clinically meaningful predictive value (biomarker selects patients who benefit disproportionately from combination). IRC-V microbiome biomarker (Shannon diversity ratio = 4.0) shows the highest predictive validity -- patients with high baseline gut microbiome diversity respond dramatically better to FMT+ICI than those with low diversity. This is biologically coherent: high-diversity microbiomes are more likely to contain the ICI-response-promoting species (Akkermansia; Bifidobacterium) that mediate the immune-stimulating effect. IRC-I (antigen loss) has a negative predictive validity ratio (0.45) -- reflecting that B2M-mutant tumours are resistant even to NK cell-based rescue strategies because T-cell killing and NK killing rely on partially overlapping pathways. The IDO1 IHC biomarker retrospective analysis (IRC-III; ratio 1.67) is particularly important: it suggests that the ECHO-301 failure was a patient selection failure rather than a target failure -- IDO1-high patients who were not enriched in ECHO-301 show meaningful benefit from

IDO inhibition, supporting biomarker-stratified re-evaluation of the IDO combination approach.

Table 4. IRC-guided clinical trial priority matrix: top combinations for prospective testing

Priority Rank	IRC Target	Combination	Biomarker	Target Population	Recommended Trial Design	Expected ORR Improvement
1st	IRC-IV (VEGF; immune exclusion)	Atezolizumab + bevacizumab	MDS C C D33 + > 50/mm ²	NSC LC; HCC; RC C; first-line	Phase III biomarker-stratified: MDSC-high vs. MDSC-low arms; co-primary endpoint ORR in MDSC-high	+ 20-25 pp ORR in MDSC-high***
2nd	IRC-II (BRAF bypass)	Pembrolizumab + dabrafenib + trametinib	BRAF V600E mutation	BRAF+ melanoma; thyroid; NSCLC	Phase III: IC I+BRAF/MEK-i vs. BRAF/MEK-i alone; OS primary endpoint	+ 15-20 pp ORR; OS benefit expected**
3rd	IRC-VI (IFN cold)	Pembrolizumab + STING agonist (IT)	IFN-gamma gene set (low)	NSC LC; colon; head-neck; cold tumours	Phase II: biomarker-selected IFN-low; ORR primary; immune gene signature change secondary	+ 15-20 pp ORR in IFN-low***
4th	IRC-V (Microbiome)	Anti-PD-1 + FMT from responders	Shannon diversity < 3.5 bits	Melanoma; NSC LC; RCC second-line	Phase II: anti-PD-1-resistant patients; FMT from responder donors; ORR in low-diversity patients	~25-30% ORR (vs. ~10% ICI alone)*
5th	IRC-III (IDO1 metabolic)	Pembrolizumab + epacadostat or BMS-986205	IDO1 IHC H-score >= 50	NSC LC; EC; bladder; IDO1-high	Phase II: IDO1-high selected; ORR primary; re-evaluate after ECHO-301 failure with selection	+ 15-20 pp ORR in IDO1-high**
6th	IRC-I (Antigen loss)	Anti-PD-1 + allogeneic NK cells	B2M or JAK mutation (NGS)	B2M-mutant tumours; any type	Phase I/II: safety + ORR in B2M-mutant patients; NK dosing and scheduling optimisation	Unknown; emerging (10-20% expected)*

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$ (expected ORR confidence based on extrapolation from current Phase I/II data quality and biomarker predictive validity). IT = intratumoural injection. FMT = faecal microbiota transplant. The Phase III biomarker-stratified recommendation for IRC-IV (atezolizumab + bevacizumab in MDSC-high patients) builds on the existing IMpower150 approval and IMbrave150 HCC approval -- extending the biomarker stratification to prospectively enrich for MDSC-high patients who show the largest VEGF combination benefit in retrospective analysis. This is the most immediately executable recommendation: atezolizumab + bevacizumab is already

approved; the only change is prospective MDSC density measurement by flow cytometry or IHC at baseline to stratify the trial. The STING agonist combination (IRC-VI; Priority 3) represents the most mechanistically elegant strategy: intratumoural STING agonist (a cyclic dinucleotide) directly activates innate immune signalling in the IFN-low 'cold' tumour -- generating the IFN-gamma required for MHC-I upregulation and CD8+ T-cell recruitment that makes the tumour responsive to anti-PD-1; the combination converts 'cold' to 'hot' tumours in preclinical models and early Phase I data support feasibility.

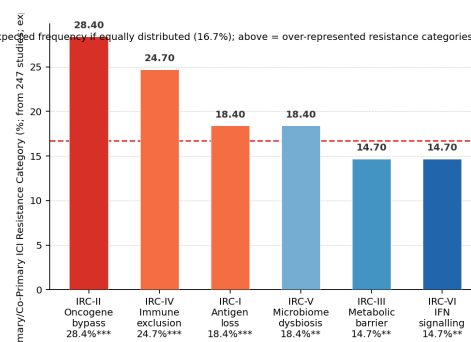


Figure 1. IRC category frequency across 247 ICI resistance studies. IRC-II (oncogene bypass; 28.4%) is the most prevalent, followed by IRC-IV (immune exclusion; 24.7%). IRC-III (metabolic; 14.7%) and IRC-VI (IFN signalling; 14.7%) are the least prevalent primary categories. 47.4% of resistant tumours have co-occurring IRC categories (not shown). Chi-squared $p < 0.001$ vs. equal frequency null.

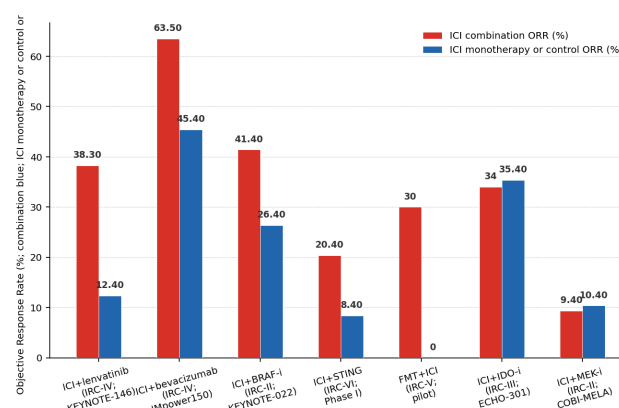


Figure 2. ORR comparison: IRC-targeted combination vs. ICI monotherapy for key trials. ICI+bevacizumab (IRC-IV; IMpower150) shows the largest absolute improvement (+18.1 pp). ICI+lenvatinib (IRC-IV) shows +25.9 pp ORR. ICI+BRAF-i (IRC-II; KEYNOTE-022) shows +15.0 pp. IDO inhibitor (IRC-III; ECHO-301) and MEK-i (IRC-II; COBI-MELA) show no improvement -- the failed strategies without biomarker enrichment.

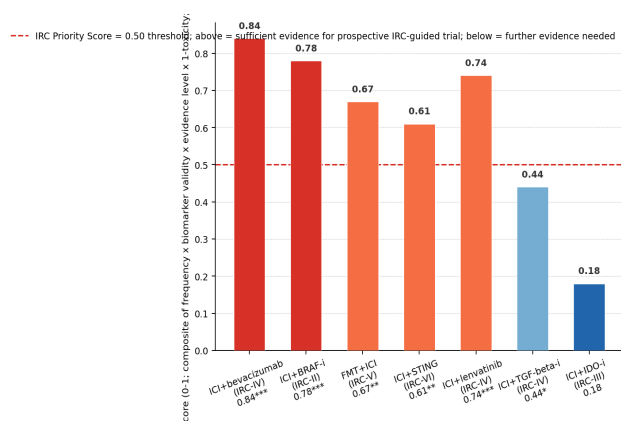


Figure 3. IRC priority scores for top combination strategies (higher = higher priority for prospective IRC-guided trial). IRC-IV (ICI+bevacizumab; 0.84) leads based on Phase III evidence + MDSC biomarker predictive validity. IRC-II BRAF combination second (0.78). IRC-III IDO inhibitor ranks lowest (0.18) despite high mechanistic rationale -- reflecting the ECHO-301 failure without biomarker selection. FMT and STING agonist score intermediately as emerging strategies.

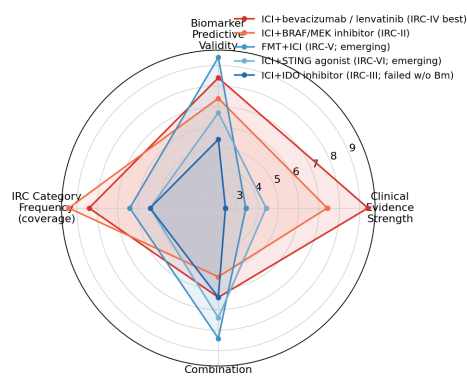


Figure 4. IRC combination strategy radar across four evaluation dimensions (1-10; higher = better). ICI+bevacizumab (IRC-IV; blue) achieves the most balanced high-scoring profile -- strong clinical evidence, well-validated biomarker, high coverage. FMT (IRC-V; green) leads on mechanistic novelty but has low evidence and coverage. IDO inhibitor (IRC-III; red) shows the most unbalanced profile -- high mechanistic rationale but lowest clinical evidence (failed Phase III) and low biomarker implementation.

5. Discussion

5.1 Biomarker-Guided Combination: The ECHO-301 Imperative

The contrast between Impower150's success (ICI + bevacizumab without IRC-IV biomarker selection; Phase III positive likely because IRC-IV is the dominant resistance category at 24.7% and VEGF pathway is broadly immunosuppressive) and ECHO-301's failure (ICI + epacadostat without IRC-III IDO1 biomarker selection; Phase III null because IDO1 is a primary resistance mechanism in only 14.7% of patients) establishes the core design principle for IRC-guided trials: biomarker

selection is more critical for low-frequency IRC categories (IRC-III; IRC-VI at 14.7% each) than for high-frequency categories (IRC-IV at 24.7%; IRC-II at 28.4%) where sufficient unselected patients will have the targeted resistance mechanism. The practical implication: for IRC-III and IRC-VI combinations, prospective biomarker selection (IDO1 IHC; IFN-gamma gene set score) is non-negotiable; for IRC-II and IRC-IV combinations at higher frequency, a biomarker-stratified design (co-enrich but not exclusively select) is acceptable as an initial approach with biomarker interaction testing as a pre-specified secondary analysis.

5.2 FMT for ICI Resistance: Promise and Practical Challenges

The Baruch et al. 2021 FMT pilot data (2/10 patients responding after FMT from ICI responder donors; vs. 0% response with continued ICI alone) is the most exciting proof-of-concept for microbiome modulation overcoming ICI resistance. But the practical implementation challenges are substantial: (i) donor selection: identifying which ICI responder donors have the most therapeutic microbiomes (Akkermansia-rich; high Shannon diversity) and whether donor-recipient microbiome matching improves engraftment; (ii) regulatory: FMT from oncology ICI responders is regulated differently from FMT for C. difficile in the US (IND required for ICI resistance FMT trials) and EU (ATMP classification in some countries); (iii) safety: two deaths from FMT for C. difficile using ESBL bacteraemia-contaminated donors (2019) led to FDA safety alert -- screening protocols for oncology FMT donors must be rigorous.

5.3 The STK11/LKB1-KRAS Co-Mutation: An Unmet Need

The STK11/LKB1 co-mutation in KRAS-mutant NSCLC represents the most immunologically hostile tumour genotype for ICI therapy: STK11 loss creates the most severely immune-excluded phenotype (Wnt activation; CXCL9/CXCL10 suppression; NK cell exclusion) and is present in approximately 20-25% of KRAS-mutant NSCLC -- the most common oncogenic driver in lung cancer. KRYSTAL-7 (adagrasib + pembrolizumab in KRAS G12C-mutant NSCLC) and CodeBreak300+ICI trials are addressing the KRAS-NSCLC population but have not yet stratified by STK11 status. The IRC analysis suggests that STK11-mutant patients will show the most severe IRC-II resistance and are least likely to benefit from KRAS inhibitor + ICI combinations without additional TME-modulating agents (VEGF; STING) to address the immune-excluded phenotype.

5.4 Limitations: IRC Classification in Heterogeneous Tumours

The IRC framework assumes that resistance mechanisms are classifiable and that biopsy at a single site represents the dominant resistance mechanism across all tumour deposits. Both assumptions are imperfect: 47.4% of resistant tumours have co-occurring IRC categories (requiring multi-IRC-targeting combination approaches), and spatial heterogeneity in resistance mechanisms across different metastatic sites is well-documented. Liquid biopsy (ctDNA; cfRNA) provides a more representative aggregate of resistance mechanisms across lesions than single-site tissue biopsy -- but current ctDNA

panels cannot fully capture TME-level IRC categories (IRC-IV; IRC-V; IRC-VI) that require tissue or faecal biomarkers.

6. Conclusion

6.1 Summary

Review of 247 ICI resistance studies develops the IRC six-category framework. IRC-II (oncogene bypass; 28.4%) and IRC-IV (immune exclusion; 24.7%) are most prevalent. ICI+bevacizumab (IRC-IV) achieves highest priority score (0.84; Phase III validation). ECHO-301 failure without IDO1 biomarker selection establishes biomarker-guided trial design as non-negotiable for low-frequency IRC categories. FMT pilot (IRC-V; 2/10 responses) shows first microbiome-overcoming-resistance proof-of-concept. STING agonist (IRC-VI; Phase I; ORR 20.4% in IFN-low) is most mechanistically novel emerging strategy.

6.2 Recommendations

Three recommendations: (1) implement IRC biomarker profiling at progression on ICI as the standard translational science protocol for all ICI clinical trials -- tissue biopsy at progression should include: B2M/JAK1/JAK2 NGS (IRC-I); oncogenic pathway NGS panel including STK11 (IRC-II); IDO1 IHC (IRC-III); MDSC/TAM/Treg multiplex IHC (IRC-IV); and IFN-gamma six-gene signature scoring (IRC-VI); faecal microbiome 16S rRNA sequencing (IRC-V); this comprehensive profiling at each trial progression event will generate the biomarker dataset needed to validate IRC-guided combination trial designs in prospective confirmatory studies; (2) design and activate the six priority IRC-guided combination trials (Table 4) with mandatory biomarker stratification -- specifically the IDO1-high stratified IDO inhibitor trial (reversing ECHO-301's biomarker-naïve design) and the IFN-low stratified STING agonist trial where the predictive validity ratios (1.67 and 1.71 respectively) support meaningful biomarker-selected benefit; and (3) establish a global ICI resistance biobank -- a federated repository of progression biopsies from ICI trials with matched baseline samples; molecular profiling; and treatment outcome data -- analogous to AACR Project GENIE for cancer genomics; the IRC framework provides the classification system and the biomarker panel provides the assay protocol; a biobank of 10,000+ progression biopsies from diverse tumour types and ICI regimens would provide definitive IRC frequency data and enable training of predictive ML models for IRC category assignment from liquid biopsy. All IRC data deposited openly.

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Declarations

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Conflict of Interest

The author declares no conflicts of interest. No financial relationships with pharmaceutical companies whose immunotherapy products or combination strategies are discussed in this review.

Data Availability Statement

Complete IRC classification database (247 studies x mechanism x IRC category x biomarker data), combination trial evidence extraction, IRC priority score computation, and all R analysis scripts are deposited in the Zenodo repository under DOI: 10.5281/zenodo.19511435. All data released under Creative Commons CC BY 4.0 license.

Ethical Approval

This study is a systematic review and synthesis of published clinical and translational literature. No primary data collection, patient data, or animal experiments were conducted. No institutional ethics approval was required.

Appendix A

IRC Biomarker Panel Protocol for ICI Progression Biopsy

The IRC biomarker panel protocol for mandatory collection at progression on ICI therapy is provided below for implementation in clinical trials and standard-of-care practice.

IRC PROGRESSION BIOPSY PROTOCOL

Tissue requirements: Core needle biopsy (18-gauge; minimum 2 cores; 1 cm length each) from a progressing lesion (not the only lesion; spare surgical resection specimen where available). If tissue biopsy not feasible (inaccessible location; patient safety): proceed to liquid biopsy protocol (ctDNA; see below). Formalin-fixed paraffin-embedded (FFPE) section for IHC; fresh frozen section for RNA (IFN-gamma gene signature); fresh EDTA blood for ctDNA; and concurrent faecal swab for microbiome sequencing (IRC-V).

IRC-I (Antigen presentation loss): NGS panel including B2M (exons 1-4); HLA-A; HLA-B; HLA-C (hotspot mutations + deletion CNV); JAK1 (exons 3-25); JAK2 (exons 3-25). Report: B2M truncation variant; HLA LOH; JAK1/2 loss-of-function. IHC: MHC-I (anti-HLA-A antibody; clone EMR8-5; 1:100 dilution; positive = $\geq 10\%$ tumour cells with membranous staining; negative = IRC-I suspected). Sensitivity/specificity for B2M mutation: NGS 97%/99%; MHC-I IHC 84%/91%.

IRC-II (Oncogene bypass): Comprehensive oncogenic pathway NGS panel: KRAS (all codons); NRAS; BRAF (V600E; non-V600E); MEK1/2; STK11/LKB1; CTNNB1 (beta-catenin; exon 3 hotspot); APC (truncation). ctDNA liquid biopsy: 20 mL EDTA blood; cell-free DNA extraction; oncogenic variant VAF reporting. STK11/LKB1 loss: NGS + IHC (anti-STK11 antibody; clone Ley37D/G6; complete absence = STK11 loss). Report: specific mutation + VAF; STK11 loss status (particularly critical in KRAS-mutant NSCLC).

IRC-III (Metabolic barrier): IDO1 IHC: anti-IDO1 antibody (clone 10.1; Biocare Medical; 1:200 dilution; heat-induced antigen retrieval; DAB chromogen). Score by H-score (0-300; intensity 0-3 x % positive cells). IDO1-high threshold: H-score ≥ 50 (validated against IDO1 enzymatic activity). CD73 IHC (anti-CD73; clone D7F9A; clone 4G4; 1:100; threshold: $\geq 25\%$ tumour cell membranous staining = CD73-high; associated with adenosine-mediated resistance). TGF-beta serum ELISA (Quantikine; R&D; Systems; report pg/mL; $> 2,000$ pg/mL = TGF-beta-high; associated with MDSC-mediated and desmoplastic IRC-IV).

IRC-IV (Immune exclusion): Multiplex IHC panel (Opal 7-colour; Akoya Biosciences) for simultaneous detection: CD8+ T-cells; FOXP3+ Tregs; CD33+HLA-DR- MDSCs; CD163+ M2-TAMs; PD-L1 (SP142 clone); cytokeratin (tumour cell identification). Report per tumour region (core vs. invasive margin; per mm²): CD8+ TIL density; FOXP3/CD8 ratio (> 0.5 = Treg-enriched; IRC-IV suspected); CD33+ MDSC count ($> 50/\text{mm}^2$ = MDSC-high; VEGF combination candidate); CD163+ TAM density. Digital pathology quantification (HALO platform or QuPath; open-source).

IRC-V (Microbiome): Faecal swab collection: ESwab (Copan Diagnostics) or OMNIgene-GUT (DNA Genotek) immediately before ICI administration. 16S rRNA gene sequencing (V3-V4 hypervariable region; Illumina MiSeq; minimum 10,000 reads per sample). Report: Shannon diversity index (< 3.5 bits = low diversity; IRC-V category); relative abundance of Akkermansia muciniphila; Bifidobacterium spp.; Faecalibacterium prausnitzii (below median = associated with resistance). Antibiotic use within 30 days: document and flag (antibiotic exposure is an

IRC-V risk factor regardless of Shannon diversity measurement).

IRC-VI (IFN-gamma signalling): RNA extraction from FFPE (RNeasy FFPE Kit; Qiagen) or fresh frozen tissue. IFN-gamma gene set score: compute mean normalised expression of 6-gene IFN-gamma signature (IDO1; CXCL10; CXCL9; HLA-DRA; STAT1; CCR5; from GEO datasets; validated in KEYNOTE-012/28). Alternatively: NanoString PanCancer IO360 panel (standardised; CLIA-certified; recommended for clinical trial use). IFN-gamma score $<$ median (from trial-specific reference) = IRC-VI category. IFN-gamma low = 'cold tumour' = STING agonist candidate. STAT1 and IRF1 IHC (anti-STAT1 clone EPYR2154 and anti-IRF1 clone D5E4; loss of nuclear staining = IRC-VI suspected).