WITHAFERIN A FROM *WITHANIA SOMNIFERA*:

A PLANT-BASED IMMUNOMODULATOR TO REDUCE ADVERSE EFFECTS OF CANCER IMMUNOTHERAPY

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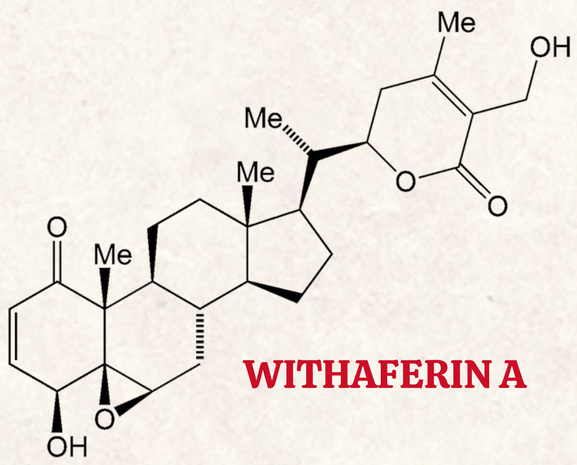
INTRODUCTION:

Cancer immunotherapy (ICIs: anti-PD-1, anti-CTLA-4)

Problem: immune-related adverse effects (irAEs)

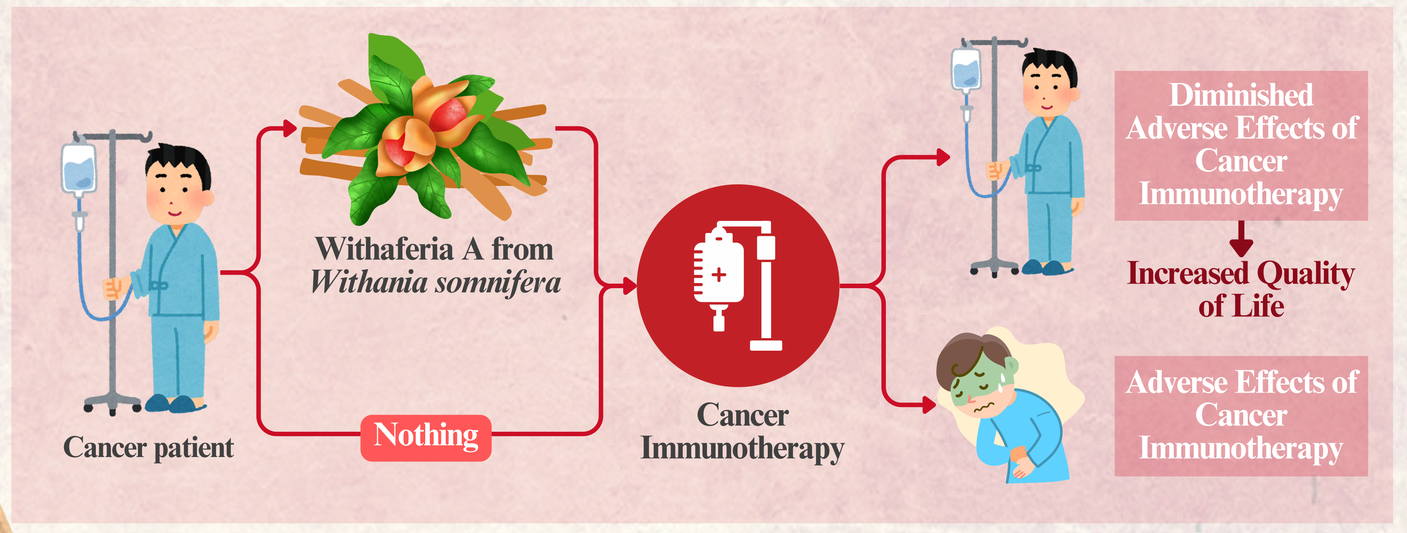
Need for complementary therapies to mitigate irAEs

Withania somnifera and Withaferin A: bioactive compound with immunomodulatory potential



WITHAFERIN A

Steroidal lactone from *Withania somnifera* (Ashwagandha)



PRECLINICAL EVIDENCE:

Model	Dosage and Regimen	Outcomes
Diabetic mice (streptozotocin-induced) with marginal mass syngeneic islet transplantation (C57BL/6 islets -> C57BL/6) – a model of islet graft stress and inflammation	WA 10 mg/kg i.p. to recipient mice, daily for 10 days post-transplant (plus donor islets pre-treated ex vivo with WA)	Enhanced islet graft survival and function: 83% of WA-treated mice achieved long-term graft survival vs. 0% in vehicle-treated controls. WA-treated grafts showed lower intragraft inflammation; serum inflammatory cytokines (IL-1β, IFN-γ) were reduced and histology showed less insulitis. Blood glucose normalized in WA group, with superior glucose tolerance tests indicating preserved islet function. These results indicate WA protected islet grafts by suppressing NF-κB-mediated inflammation
Mouse **acute graft-versus-host disease (GVHD)** model (allogeneic bone marrow transplant from C57BL/6 -> BALB/c) – model for T cell–mediated autoimmunity (similar to irAE conditions)	WA 4 mg/kg i.p. daily, starting day 0 for 14 days post-BMT	**Prevention of acute GVHD:** WA-treated mice had markedly **lower clinical GVHD scores** (mean score ≤2 vs. ≥6 in controls, on a 0–10 scale) and improved survival at 30 days (70% vs. 20% in controls). Mechanistically, WA treatment led to **reduced splenic T-cell activation** (reduced CD69^+ T cells) and proliferation in vivo, correlating with **lower serum IFN-γ and TNF-α levels**. Lymphoid organ histopathology showed less T cell infiltration and tissue damage. WA's effects were associated with **downregulation of AKT/mTOR signaling** in donor T cells, curbing their expansion. This study demonstrated WA can **mitigate a severe T cell–driven immunopathic condition** analogous to immunotherapy-related autoimmunity.
Allogeneic islet transplantation in diabetic mice (BALB/c islet donor → C57BL/6 recipient) under minimal immunosuppression – model of transplant rejection similar to checkpoint inhibitor-induced organ autoimmunity	WA 5 mg/kg i.p. to both donors (during islet isolation) and recipients (daily for 1 week post-transplant)	**Prolonged islet allograft survival:** 80% of WA-treated recipients maintained graft function long-term vs. 40% in control mice on low-dose tacrolimus. WA did not compromise graft acceptance – in fact, it performed better than tacrolimus in this model. Immune analyses showed suppressed T-cell proliferation in WA-treated mice and significantly impaired dendritic cell maturation, evidenced by lower CD83 and MHC-II expression on DCs. Inflammatory mediators in the graft microenvironment were reduced: exosomes from WA-treated islets contained significantly less IL-6, MCP-1, and iNOS. hese findings highlight WA's potential as an adjunct to control immune reactions (T-cell and DC-driven) without hindering the desired graft/tumor outcomes.

Sources:
(1) Kenjiro Kumano et al. Withaferin A inhibits lymphocyte proliferation, dendritic cell maturation in vitro and prolongs islet allograft survival, Nature Portfolio, Vol. 11, no. 1 pp. 1 – 11, https://doi.org/10.1038/s41598-021-90181-y
(2) Mehta M, Gohil D, Khattry N, Kumar R, Sandur S, Sharma D, Checker R, Agarwal B, Jha D, Majumdar A, Gota V. Prevention of acute graft-versus-host-disease by Withaferin a via suppression of AKT/mTOR pathway. Int Immunopharmacol. 2020 Jul;84:106575. doi: 10.1016/j.intimp.2020.106575. Epub 2020 May 13. PMID: 32416453.
(3) SoRelle JA, Itoh T, Peng H, Kanak MA, Sugimoto K, Matsumoto S, Levy MF, Lawrence MC, Naziruddin B. Withaferin A inhibits pro-inflammatory cytokine-induced damage to islets in culture and following transplantation. Diabetologia. 2013 Apr;56(4):814-24. doi: 10.1007/s00125-012-2813-9. Epub 2013 Jan 15. PMID: 23318585.

KEY IMMUNOMODULATORY MECHANISMS OF WITHAFERIN A

1 — *Inhibition of NF-κB Signaling*

- *WA targets the NF-κB pathway by covalently modifying cysteine-179 on IKKβ.*
- *This prevents IKKβ activation and stops phosphorylation/degradation of IκB.*
- *Result: NF-κB stays trapped in the cytoplasm, unable to trigger inflammatory gene transcription.*
- *In THP-1 monocytes, WA blocks NF-κB p65 nuclear translocation after stimulation.*
- *Shown to inhibit NF-κB activity across various cell types (immune, endothelial, epithelial).*
- *Reduces downstream inflammatory cascades contributing to irAEs*

Sources:
SoRelle JA, Itoh T, Peng H, Kanak MA, Sugimoto K, Matsumoto S, Levy MF, Lawrence MC, Naziruddin B. Withaferin A inhibits pro-inflammatory cytokine-induced damage to islets in culture and following transplantation. Diabetologia. 2013 Apr;56(4):814-24. doi: 10.1007/s00125-012-2813-9. Epub 2013 Jan 15. PMID: 23318585.

2 — *Modulation of T-Cell Responses*

- *WA's NF-κB inhibition leads to lowered cytokine release.*
- *In vitro studies show reduced IL-6 and TNF-α production in immune cells after LPS stimulation.*
- *In endothelial cells, WA suppresses palmitate-induced IL-6 and TNF-α by blocking IKKβ/NF-κB.*
- *In macrophages, WA reduces both mRNA and protein levels of IL-6 and TNF-α.*
- *In cancer models, WA reduces IL-1β, IL-6, and TNF-α expression elevated 3–5 times by tumors ($p<0.001$).*
- *In mouse hepatitis models, WA decreases systemic TNF and IL-1, protecting against cytokine storms.*

Sources:
Xia Y, Wang P, Yan N, Gonzalez FJ, Yan T. Withaferin A alleviates fulminant hepatitis by targeting macrophage and NLRP3. Cell Death Dis. 2021 Feb 11;12(2):174. doi: 10.1038/s41419-020-03243-w. PMID: 33574236; PMCID: PMC7878893.

3 — *Suppression of Pro-inflammatory Cytokines (IL-6, TNF-α, etc.)*

- *WA inhibits T-cell and B-cell proliferation at ≤5 μM without causing cell death.*
- *Downregulates early activation markers on T-cells (CD25, CD69, CD71, CD54).*
- *Reduces Th1 (IL-2, IFN-γ) and Th2 (IL-4, IL-5) cytokine secretion.*
- *In mixed lymphocyte reactions, WA blocks T-cell proliferation.*
- *Prevents overactive T-cell responses that lead to autoimmune-like irAEs (e.g., colitis, hepatitis).*
- *Does not cause apoptosis, indicating a reversible regulatory effect rather than full immunosuppression.*

Sources:
Gambhir L, Checker R, Sharma D, Thoh M, Patil A, Degani M, Gota V, Sandur SK. Thiol dependent NF-κB suppression and inhibition of T-cell mediated adaptive immune responses by a naturally occurring steroidal lactone Withaferin A. Toxicol Appl Pharmacol. 2015 Dec 1;289(2):297-312. doi: 10.1016/j.taap.2015.09.014. Epub 2015 Sep 25. PMID: 26408225.

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