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KAWASAKI INSTITUTE OF INDUSTRIAL PROMOTION
Innovation Center of NanoMedicine

CHANGE
Center of Healthy longevity And Nursing innovation with Global Ecosystem

Advancing Idiopathic Pulmonary Fibrosis treatment with PROTAC Nanomedicine

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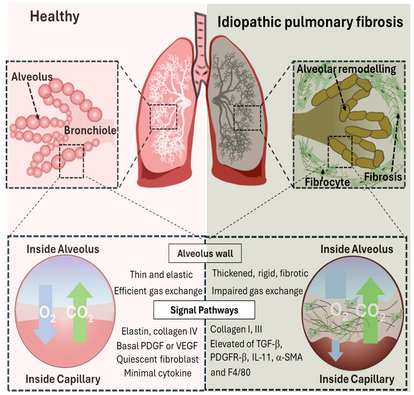
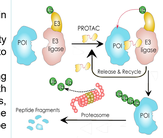
Proteolysis Targeting Chimeras (PROTAC)



Idiopathic pulmonary fibrosis (IPF): is a chronic, progressive, and ultimately fatal fibrotic lung disease with a median survival time of 3-4 years from diagnosis. **Proteolysis Targeting Chimeras (PROTAC)** BRD4 degrader, **ARV-825** attenuates fibrosis and ameliorates lung function in an experimental murine model of lung fibrosis.

★ Why PROTACs?

1. High selectivity
2. Improved efficacy using protein degradation pathways
3. Even with weak binding, activity is amplified, → Undruggable to druggable
4. Effective against weakly binding proteins (c-myc, K-ras, etc.) with shallow binding pockets, accounting for 80% of the proteins that cannot be formulated

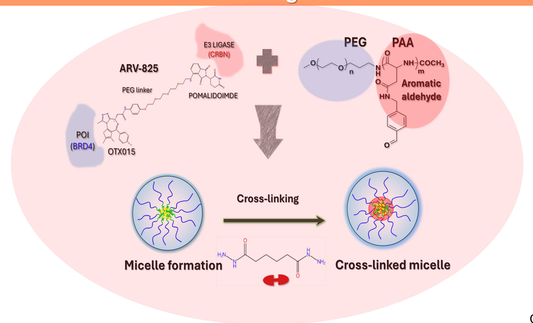


★ Problems with PROTACs

1. Inefficient uptake into the cytoplasm
2. Target and E3 ligase selectivity
3. Stability and metabolism, Toxicity
4. Difficulty in control

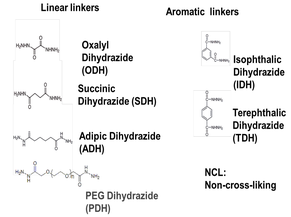
→ DDS Platform

Design of PROTAC nanomedicine targeting IPF



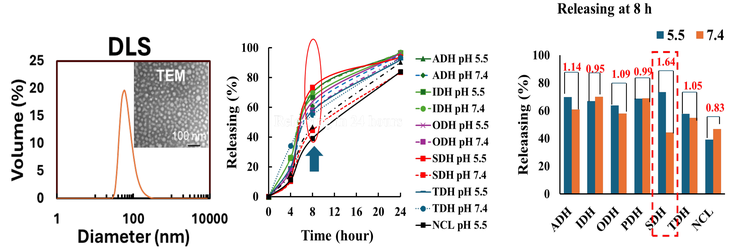
The aromatic aldehyde contained polymers and ARV-825 were used for the preparation of micelle with self-assembly and following by a cross-linking process. The structure contained a block copolymer having a hydrophilic segment from polyethylene glycol (PEG), an aromatic hydrophobic block segment contained aldehyde as a crosslinking block segment, with a PROTAC molecule encapsulated by the block copolymer.

Evaluate cross-linking efficiency of linkers



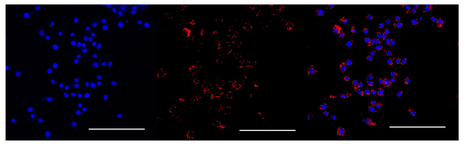
Chemical structures of representative dihydrazides. These molecules differ in backbone length, rigidity, and functional group positioning, which may influence their reactivity and spatial compatibility with the components of the micelle. They are systematically compared to identify the most suitable linker for micelle cross-linking, aiming to enhance micellar stability, drug encapsulation efficiency, and controlled release performance.

Size and releasing profiles



The nanomedicine exhibited a uniform size (~70 nm), high ARV-825 encapsulation efficiency (>85%), and excellent colloidal stability. In vitro drug release profiles of micelles cross-linked with different dihydrazides (SDH, ADH, IDH, TDH, ODH) compared to non-cross-linked micelles (NCL), evaluated at pH 7.4 and pH 5.5. Micelles cross-linked with succinic dihydrazide (SDH) exhibited a notably faster drug release at acidic pH (5.5) than at physiological pH (7.4), suggesting pH-responsive behavior. This pH-dependent release was more pronounced for SDH-cross-linked micelles compared to other cross-linkers and the non-cross-linked control, highlighting SDH as a promising candidate for stimuli-responsive drug delivery. Hereafter, ARV-825 will be mentioned as ARV.

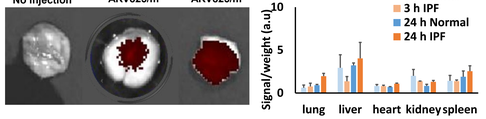
Cell uptake



Cellular uptake of Alexa Fluor 647-labeled ARV-825 micelles by RAW264.7 macrophage cells was analyzed by fluorescence microscopy. Micelles were efficiently internalized, showing strong intracellular fluorescence signals after 3 hours of incubation. This indicates rapid and effective uptake by macrophages, supporting their potential for targeted delivery in immune-related or inflammatory environments. **Blue: DAPI, red: Alexa 647 ARV-825 crosslinking micelles.**

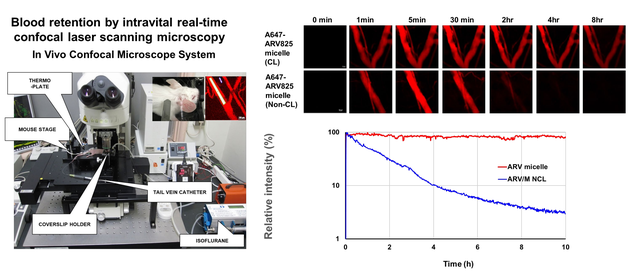
Lens: 40X, scale bar: 100 μm.

Biodistribution



Biodistribution analysis of Alexa Fluor 647-labeled ARV-825 micelles in normal and IPF mice using IVIS imaging at defined time points post-injection. The nanomicelles exhibited markedly higher accumulation in IPF lung tissue compared to normal lung, indicating enhanced accumulation in the fibrosis area. This preferential localization suggests that the micellar formulation improves biodistribution and potentially prolongs drug retention in fibrosis lung tissue, which is advantageous for treating pulmonary diseases such as IPF.

Blood circulation

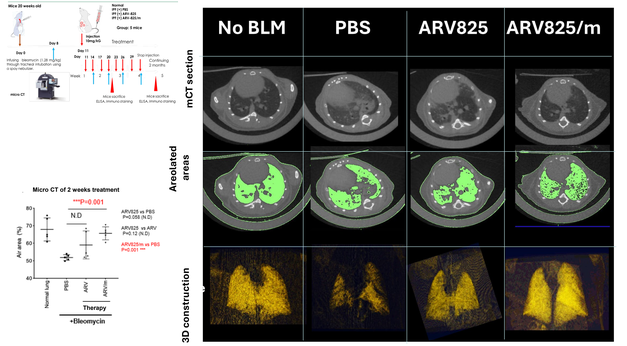


In vivo blood circulation assessment of Alexa Fluor 647-labeled micelles using confocal intravital microscopy of the ear vasculature. Cross-linked micelles exhibited prolonged vascular retention, with strong fluorescence signals detectable for over 10 hours post-injection. In contrast, non-cross-linked micelles showed a more rapid clearance from circulation. These results highlight the enhanced stability and extended circulation time conferred by cross-linking, supporting their suitability for sustained drug delivery applications.

Conclusions and perspectives

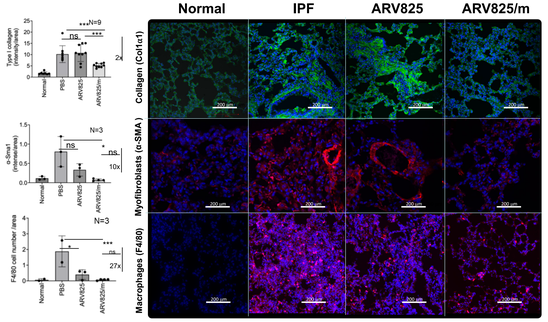
In summary, we engineered a PROTAC-loaded nanomicelle system with well-defined physicochemical properties, demonstrating pH-sensitive release and enhanced pulmonary accumulation, which translated into superior therapeutic efficacy in a murine IPF model compared to free ARV-825. The nanomicelle treatment effectively mitigated fibrosis, as evidenced by significant reductions in collagen deposition, myofibroblast activation, macrophage infiltration, and senescence markers, alongside notable preservation of lung architecture and function. These findings underscore the potential of nanocarrier-mediated PROTAC delivery to overcome current limitations of small-molecule degraders in fibrotic lung disease, offering a precision strategy to modulate pathogenic protein networks. Future work will focus on mechanistic elucidation of intracellular trafficking and degradation pathways, long-term safety assessment, and translational studies to advance this platform toward clinical application in IPF and related fibrotic disorders.

In vivo anti-fibrotic effects



Representative micro-CT images of mice at 2 weeks post-injection of therapeutic agents in the idiopathic pulmonary fibrosis (IPF) model. Grayscale images depict overall lung structure, with green highlighting aerated (areolated) regions and yellow representing 3D lung reconstructions. Mice treated with ARV825-loaded micelles exhibited a marked reduction in fibrotic lesions and a notable improvement in lung architecture, with expanded aerated areas comparable to those of healthy controls. In contrast, mice receiving PBS or free ARV-825 showed extensive fibrotic changes and diminished aeration, underscoring the enhanced therapeutic efficacy of the micelle-based delivery system.

Immunostaining



Immunohistochemical analysis of lung tissue sections stained with antibodies against Collagen I, α-SMA, and F4/80 to evaluate fibrosis and macrophage infiltration in the IPF mouse model. Mice treated with ARV825-loaded micelles showed a significant reduction in collagen deposition, myofibroblast activation (α-SMA), and macrophage presence (F4/80) compared to PBS and free ARV825-treated groups. Staining patterns in the micelle-treated group were comparable to those in healthy control mice, indicating strong suppression of fibrosis and inflammation. Quantitative analysis confirmed the differences were statistically significant, supporting the enhanced therapeutic potential of ARV825 micelles.

References

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Acknowledgement

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