

Supplementary Information

Topical Small-molecule Granzyme B Inhibitor Improves Remodeling in A Murine Model of Impaired Burn Wound Healing

Running Title: Novel Therapy for Burn Injury and Impaired Healing

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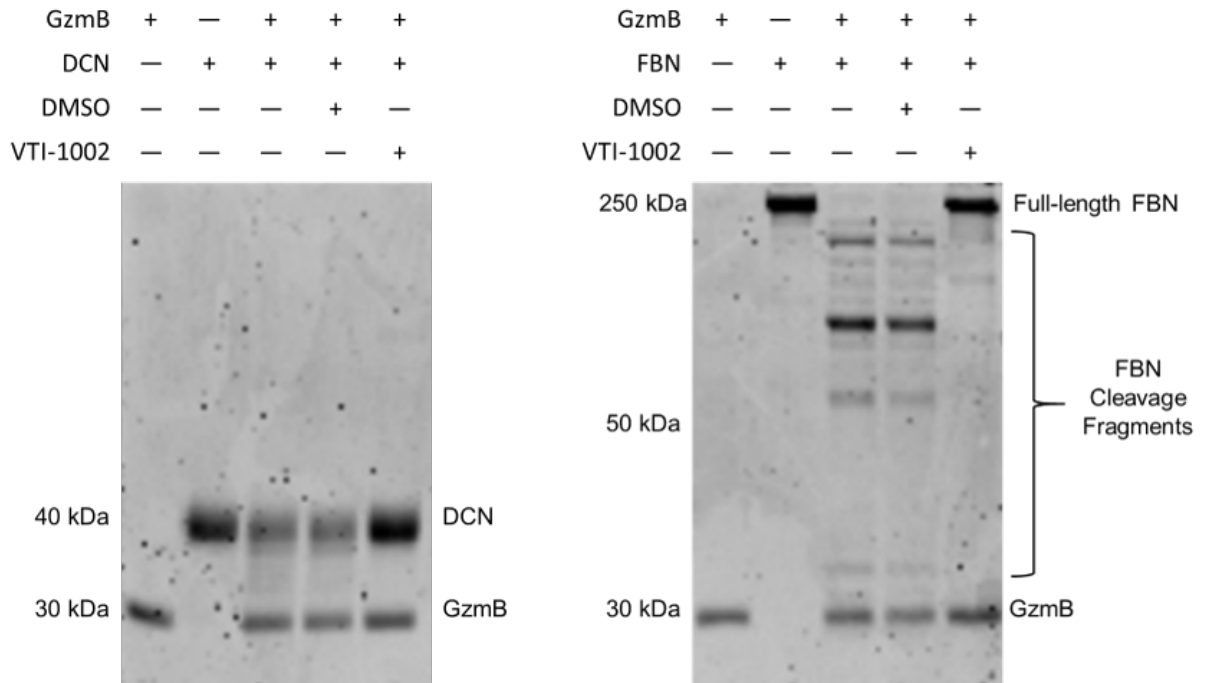
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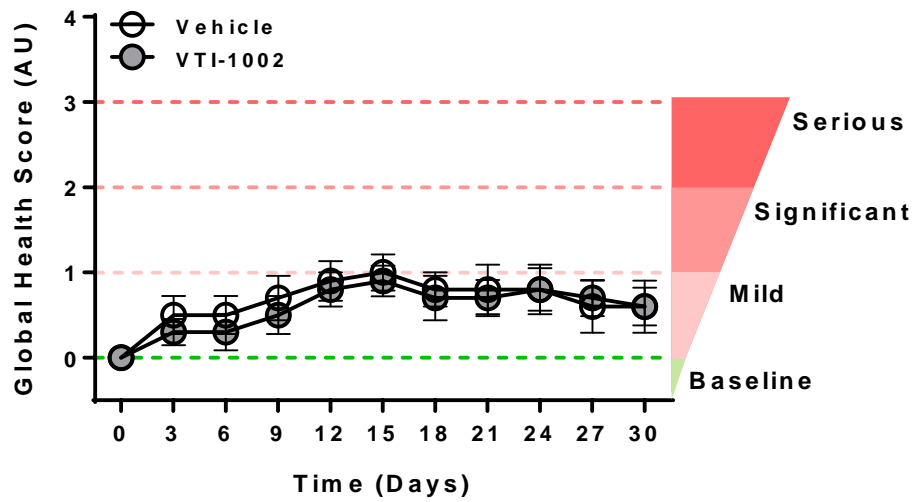
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Supplementary Figure 1. *In vitro* Characterization of VTI-1002 activity. VTI-1002 abolishes GzmB-mediated ECM cleavage. (left) GzmB-mediated DCN cleavage assay; (right) GzmB-mediated FBN cleavage assay.



Supplementary Figure 2. Topical VTI-1002 gel treatment does not affect global health. Comparison of global health score over the 30-day healing period between vehicle control group and VTI-1002 treatment group (n = 10 per group). Global health score is the sum of scores of each health observation category (**Supp. Table 2**). Score 0 indicates the baseline health (normal); Score 1 indicates mild health impact; Score 2 indicates significant health impact; Score 3 indicates serious health impact (humane endpoint).

Supplementary Table 1: Plasma level of VTI-1002 after topical application

| Time Point | 4 hrs | | | 6 hrs | | | 24 hrs | | |
|-----------------------|--------------|----------|----------|--------------|----------|----------|---------------|----------|----------|
| Sample # | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Plasma (ng/mL) | 1.6 | BLOQ | BLOQ | 7.6 | 7.4 | BLOQ | ND | BLOQ | 3.4 |

BLOQ: Below the limit of quantitation (1 ng/mL in plasma)

ND: Not detected.

Supplementary Table 2: Global Health Score Reference Chart

| Health Observation Categories | Health Score | | | |
|-------------------------------|------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------|
| | 0 (Baseline health) | 1 (Mild) | 2 (Significant) | 3 (Serious, Humane endpoint) |
| Body weight | ↓weight by 0-4% | ↓weight by 5- 9% | ↓weight by 10-14% | ↓weight over 15% |
| Wound site | Occasional scratching | Redness or scaling | Skin inflammation, drug-induced rash | Severe skin inflammation, ulceration |
| Behavior | Intermittent stereotypic behaviour during cage changing; BAR (Bright, Alert, Responsive) | Slightly slow moving; still interested in environment; QAR (Quiet, Alert, Responsive) | Less interested in the environment, interacts less with cage mates, disregards observer; when nudged, reluctantly moves away. Occasional abnormal gait | Isolated from cage mates, minimally active; does not readily move when cage disturbed. When nudged reluctantly moves. Limping or “tip-toed” gait. |
| Dehydration | N/A | Mildly sunken eyes (appear >75% open). | Skin tent > 2 seconds (decrease in skin elasticity). Sunken eyes (appear half closed). | Skin tent > 5 seconds. Completely closed or severely sunken eyes; tail feels square. Cool to touch. |
| Elimination (Feces, Urine) | Soft but formed feces, hydration normal | Daily paste-like feces, hydration normal | Daily watery diarrhea, occasionally formed, soft feces, increased urine output. Small area of fecal anus staining, urine scalding. Hydration normal | Watery diarrhea or increased urination resulting in dehydration; larger area of fecal staining, urine scalding, fecal smearing on walls |
| Respiration | Occasional increased rate | Subtle change in rate or effort | Obvious change in rate or effort | Reduced rate, irregular rhythm; |

| | | | | |
|------|------------------------------------------------|-----------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------|
| | after activity; normal rhythm and effort | with activity; normal at rest; regular rhythm | with activity and at rest; occasional irregular rhythm; small amount of nasal discharge, sneezing | requires effort to breathe (abdominal effort); noisy breathing; nasal discharge affecting breathing |
| Pain | None | N/A | Facial grimace: narrow or closed eyes, bulge on top of nose, cheek bulge, ears back or flat, whiskers pointing back or “standing out on end” Other: muscle twitching or flinching, staggering, back stretch, abdominal writhing or pressing | Persistent signs of pain that interfere with normal functions or cannot be alleviated |