

Original article

First-in-Human Phase I Study of a Single-Dose Extract of *Gratiola officinalis* L. (AV-22) in Patients with Advanced Genitourinary Cancers: Safety and Tolerability

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Abstract: This first-in-human Phase I study assessed the safety and tolerability of the industrial-grade batch of AV-22 dry medicinal extract (*Gratiola officinalis* L.) (Developing organization: Saratov State Medical University named after V.I. Razumovsky, Ministry of Health of Russia).

Objective — To evaluate the safety, tolerability, and maximum tolerated dose (MTD) of a single oral dose of AV-22 in patients with advanced genitourinary cancers.

Material and Methods — An open-label, single-center, dose-escalation study was conducted using the classic 3+3 design [12]. Twenty patients with advanced genitourinary cancers were sequentially enrolled in five dose cohorts (125, 250, 375, 500, and 625 mg). Adverse events were graded using the Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. Tumor response was assessed at day 28 using Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. Pharmacokinetic analyses quantified plasma concentrations of verbascoside, cucurbitacin D, cucurbitacin E, and cucurbitacin I after oral administration. Concentrations were quantified using a validated method of high-performance liquid chromatography with a mass-selective detector (HPLC-MS/MS) with a linear range of 1-1000 ng/mL (lower limit of quantification (LLOQ): 1 ng/mL). Serial blood samples for pharmacokinetic analysis were collected before and after drug administration at designated time points: 0.5, 1, 3, 6, 9, 12, 15, 18, 24, and 36 hours, as well as on days 7, 14, and 28 (all samples obtained in the morning after an overnight fast).

Results — All 20 patients completed the observation protocol (mean age =65.8±9.6 years). No dose-limiting toxicity (DLT) or serious adverse events were reported, and the maximum tolerated dose (MTD) was not reached at any dose level up to 625 mg. Plasma concentrations of all analyzed active components (verbascoside, cucurbitacins D, E, and I) were below the lower limit of quantification (LLOQ=1 ng/mL) at all sampling time points, precluding the calculation of standard pharmacokinetic parameters (e.g., AUC, C_{max}, T_{max}, t_{1/2}). Statistically significant changes in individual laboratory parameters (e.g., hemoglobin, thrombin time, and total bilirubin) were small in magnitude and were not accompanied by clinically significant deviations. Follow-up computed tomography or magnetic resonance imaging (CT/MRI) was performed in all patients on day 28: all 20 patients (100%) achieved stable disease, with no partial or complete responses or progressive disease.

Conclusion — A single 125-625 mg dose of AV-22 exhibited a favorable safety profile and tolerability in patients with advanced genitourinary cancers. The data obtained justify further studies with multiple doses and extended biomarker monitoring.

Keywords: *Gratiola officinalis* L., phase I study, dose escalation, safety, tolerability, genitourinary neoplasms.

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Introduction

Genitourinary malignancies constitute a major proportion of global cancer incidence and mortality; particularly, in advanced stages, they substantially impair survival and quality of life. International Agency for Research on Cancer (IARC) GLOBOCAN 2022 estimates indicate 20 million new cancer cases and 9.7 million cancer-related deaths worldwide, underscoring the immense burden of cancer and the urgent need for novel therapeutic and supportive strategies — especially for patients with advanced genitourinary cancer [1].

In the Russian Federation, common genitourinary tumors also remain a major clinical and societal challenge because of high

incidence, a growing cohort of long-term survivors who require chronic care, and extensive use of systemic therapy and prolonged follow-up. National cancer care statistics reveal persistent strain on health-care infrastructure and highlight the imperative to expand safe, well-tolerated therapeutic options for vulnerable subpopulations, including patients with polymorbidity and limited tolerance for intensive regimens [2].

Current systemic therapies for common genitourinary tumors encompass hormonal, chemotherapeutic, targeted, and immunological approaches. While these modalities have demonstrated measurable improvements in survival, significant toxicity frequently offsets their clinical benefits. For instance, the addition of docetaxel

to androgen deprivation therapy in metastatic hormone-sensitive prostate cancer improves overall survival but concurrently increases the risk of grade ≥ 3 adverse events, including neutropenia and severe fatigue [3].

The toxicity of combination regimens poses a particular challenge. Meta-analyses show that even with a potential enhancement of antitumor response – for example, with combinations of immunotherapeutic agents in urothelial carcinoma – there is a significant increase in the incidence of severe adverse events [4].

These limitations underscore the need for novel anticancer agents with an improved therapeutic index, particularly those suitable for use in patients with complex medical histories – a scenario that mirrors real-world clinical practice. In this context, natural compounds, which have historically served as the foundation of oncological pharmacotherapy, remain of considerable interest. A significant proportion of modern chemotherapeutic agents are either plant-derived or based on plant compounds. Current analyses indicate that natural molecules remain one of the most productive sources for the discovery of new drug candidates, including anticancer agents [5].

One promising source of new biologically active compounds is *Gratiola officinalis* L. The biological activity of this plant is associated with various classes of compounds, including iridoids, flavonoids, phenolic acids, apigenin glycosides, and triterpene cucurbitacins. These components have demonstrated antiproliferative and proapoptotic properties. Cucurbitacins are considered promising molecules with antitumor potential, as reflected in patent reviews and mechanistic studies. However, issues of toxicity and pharmacokinetics (absorption, distribution, metabolism, and excretion) have also been highlighted, limiting the direct translation of preclinical results into clinical practice without careful early-phase safety assessment in humans [6, 7].

Gratiola officinalis L. extract (AV-22) is an active pharmaceutical substance of plant origin, produced at a Russian GMP-certified pharmaceutical facility. This extract is considered a promising source of biologically active compounds with antitumor potential. Modern chemical and biological studies of flavonoid-containing fractions of *Gratiola officinalis* L. extract have confirmed the presence of components with antitumor activity and characterized the spectrum of biological action, forming the basis for further translational development [8].

To justify the transition to clinical studies, preclinical data demonstrating the direct antitumor activity of AV-22 on human tumor cell cultures are of fundamental importance.

Preclinical studies by Saratov State Medical University named after V.I. Razumovsky, Ministry of Health of Russia, demonstrated its antitumor activity. Initial studies showed a pro-apoptotic effect of the flavonoid-containing extract on human kidney cancer cell lines (Caki-1, SN12c) [9]. A subsequent study on an expanded panel of cell lines – including PC-3 (prostate cancer), A498 (kidney cancer), and other solid tumors – using flow cytometry, established that *Gratiola officinalis* L. extract induces apoptosis in tumor cells primarily via the mitochondrial pathway with caspase activation, causes cell cycle arrest in the G2/M phase, reduces the proliferation rate of urogenital neoplasms, and inhibits angiogenesis [10].

In immunodeficient mice, oral administration of AV-22 as monotherapy (500 mg/kg) resulted in a 59% inhibition of tumor growth (by volume) by day 21 post-transplantation. In combination

therapy with docetaxel, use of half the dose of AV-22 (250 mg/kg) produced a synergistic effect, increasing the tumor growth inhibition index (TGI) to 84%. The antitumor effect of the 250 mg/kg dose persisted for 14 days after discontinuation of the drug. Acute and subacute toxicity studies showed that at the therapeutically effective dose (110 mg/kg), AV-22 has no adverse effects on key body systems. The lethal dose (LD50) in experimental models was 5000 mg/kg. According to GOST 12.1.007-76 classification, the drug solution was classified as a Class 3 hazard (“moderately hazardous substances”) based on acute toxicity parameters. These data support its further development as an antitumor agent.

In parallel, a study was conducted to optimize the extract’s dosage form, thereby enhancing its stability and controllability of action. In particular, experimental data on the microencapsulated form of the flavonoid-containing extract show that its biological activity is preserved, opening prospects for the development of reproducible and technologically feasible drugs based on it [11].

Based on these data – confirming the therapeutic efficacy and acceptable preclinical safety profile – a decision was made to advance to the clinical phase of development.

The relevance of this work is determined by several factors: the high prevalence and mortality of tumors of the genitourinary system at the population level; the ongoing clinical challenge of resistance and toxicity in multi-stage systemic therapy for advanced-stage disease; the scientifically grounded interest in natural sources of antitumor compounds; the availability of preclinical data on the proapoptotic activity of the flavonoid-containing extract of *Gratiola officinalis* L. against human tumor cell lines; and the need to obtain primary clinical data on the safety and tolerability of AV-22 following single-dose administration in patients who have exhausted standard treatment options. Against this background, the clinical evaluation of the safety and tolerability of a standardized industrial preparation based on *Gratiola officinalis* L. extract represents a logical next step in translating preclinical findings into clinical application.

Study objective. To evaluate the safety, tolerability, and to determine the maximum tolerated dose (MTD) of a single oral dose of AV-22 tablets in patients with locally advanced or metastatic genitourinary cancers.

Material and Methods

Study design. A pilot, open-label, single-center, prospective Phase I study with sequential dose escalation using the classic 3+3 design [12].

Ethical aspects. The study was conducted at V.I. Razumovsky Saratov State Medical University (Saratov, Russia). The protocol was approved by the Ethics Committee of V.I. Razumovsky Saratov State Medical University (Protocol No. 16, dated August 4, 2023). All study participants provided written informed voluntary consent. The study was conducted in accordance with the principles of ICH Good Clinical Practice (GCP E6 (R2/R3)) and the Declaration of Helsinki of the World Medical Association [13]. In line with ICH S9 guidance for anticancer pharmaceuticals, AV-22 was evaluated in patients with advanced cancer rather than in healthy volunteers [16].

Study participants. Patients aged ≥ 18 years with morphologically verified locally advanced or metastatic genitourinary cancer (involving the kidney, renal pelvis, ureter, bladder, urethra, prostate, testis, or penis). This approach is

justified for the initial safety assessment of an agent targeting a common biological mechanism – such as induction of apoptosis via the mitochondrial pathway – that may be broadly applicable across tumor types and facilitates optimal patient recruitment during early-phase development [14].

Key inclusion criteria:

- Eastern Cooperative Oncology Group (ECOG) performance status of 0–2
- Satisfactory bone marrow function: neutrophils $\geq 1.5 \times 10^9/L$, platelets $\geq 75 \times 10^9/L$, hemoglobin $>80 \text{ g/L}$
- Satisfactory liver function: total bilirubin $\leq 1.5 \times$ upper limit of normal (ULN), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) $\leq 2.5 \times$ ULN
- Satisfactory renal function: creatinine clearance $>30 \text{ mL/min}$ (calculated by Cockcroft–Gault equation)

Exclusion criteria:

- Known hypersensitivity to any component of AV-22
- Pregnancy or lactation
- Presence of another active malignant neoplasm
- Severe, uncontrolled concomitant or infectious disease
- Mental or substance use disorders that could interfere with protocol adherence

Investigational Medicinal Product, Dose, and Method of Administration

AV-22 is a tablet formulation containing 125 mg of standardized dry extract of *Gratiola officinalis* L. Five planned dose levels corresponded to the oral administration of 1, 2, 3, 4, or 5 tablets per day, delivering 125, 250, 375, 500, or 625 mg of extract, respectively. Patients received a single oral dose in the morning on an empty stomach. Dose escalation followed the 3+3 design. Each cohort initially enrolled three patients. If no DLT occurred during the observation period, enrollment proceeded to the next higher dose level. If one DLT occurred, the cohort was expanded to six patients. Dose escalation was halted when two or more patients experienced DLT at a given dose level; in such cases, the preceding dose level was designated as the maximum tolerated dose (MTD). Decisions regarding dose escalation were made by the Independent Safety Monitoring Committee based on a comprehensive review of all adverse events.

Study Procedures and Safety Assessment

The screening period lasted up to seven days before drug administration (Day 1). On Day 1, assessments were conducted before and one hour after drug administration, including vital signs, clinical examination, laboratory tests, standard 12-lead ECG, and recording of adverse events. Inpatient observation continued for up to three days (Days 1–3). Follow-up visits were scheduled on Days 7, 14, and 28. At each visit, vital signs were reassessed, adverse events and serious adverse events (SAEs) were recorded, and a physical examination, ECG, and a standard panel of laboratory tests (clinical and biochemical blood tests, urinalysis, and coagulogram) were performed.

Pharmacokinetic evaluation was performed by measuring plasma concentrations of verbascoside, cucurbitacin D, cucurbitacin E, and cucurbitacin I following oral administration of the study drug. Concentrations were determined using a validated method of high-

performance liquid chromatography with a mass-selective detector (HPLC-MS/MS), with a quantification range of 1–1000 ng/mL (lower limit of quantification: 1 ng/mL). Blood samples for pharmacokinetic analysis were collected before drug administration and at multiple designated post-dose time points: 0.5, 1, 3, 6, 9, 12, 15, 18, 24, and 36 hours, as well as on Days 7, 14, and 28 (all samples collected in the morning under fasted conditions).

Endpoints

The primary endpoint was the safety and tolerability of a single oral dose of AV-22, assessed by the frequency and severity of adverse events, including DLT, as well as changes in laboratory parameters, electrocardiograms (ECGs), and vital signs. The severity of AEs was graded according to the Common Terminology Criteria for Adverse Events, version 4.03 (CTCAE v4.03). DLT was defined as protocol-specified adverse events of grade 3 or 4 severity (with a possible, probable, or definite causal relationship to AV-22 administration) occurring within the first 7 days following a single dose.

Sample Size Determination

As this was a Phase I pilot study designed primarily to evaluate safety rather than to formally confirm efficacy, no formal sample size calculation based on statistical power was performed. The target sample size of 20 patients was selected in accordance with established methodology for early-phase oncology trials. This number was determined using the standard 3+3 dose escalation design, a widely accepted approach for identifying the maximum tolerated dose (MTD) and characterizing the safety profile of novel therapeutic agents [12, 15]. Considering the five planned dose levels and the typical cohort size of three to six patients per level, a total of up to 20 participants was considered sufficient to complete dose escalation and characterize the initial safety profile of AV-22. From an ethical perspective, increasing the number of participants beyond the minimum required by the standard design for studying a new compound at an early stage would be unjustified.

Statistical Analysis

Statistical analysis was primarily descriptive and exploratory in nature. The normality of quantitative variables was assessed using the Shapiro–Wilk test. Based on the test results and the small sample size, non-parametric methods were selected for the analysis. Continuous variables are presented as mean \pm standard deviation (SD), as well as median and interquartile range (IQR). Categorical variables are presented as the number of observations and percentage (n, %).

To evaluate intra-individual changes in laboratory parameters and vital signs across visits, the non-parametric Friedman test for repeated measures was applied. For variables demonstrating statistically significant changes in the Friedman test ($pFDR < 0.05$), pairwise comparisons against baseline (Screening/Day 1) were conducted using the Wilcoxon signed-rank test, with multiple comparisons corrected using the Benjamini–Hochberg procedure [17].

Safety analyses also included a descriptive assessment of the frequency of adverse events and laboratory abnormalities by dose cohort.

For exploratory research purposes, pairwise Spearman's rank correlation coefficients were computed to assess associations between (1) baseline (screening) values of all parameters and (2) their respective changes from baseline to Day 28 (Δ Day 28). Associations between dose level and Δ Day 28 were evaluated similarly. In all correlation analyses, the Benjamini-Hochberg method (FDR) was applied to control for multiple comparisons. Statistical analyses were performed using Spotfire Statistica v.12.0 (formerly TIBCO Statistica; Cloud Software Group, Palo Alto, CA, USA).

Results

The study included 20 patients divided into five sequential dose cohorts. Demographic and baseline characteristics were balanced across groups. The cohorts receiving 125 mg, 375 mg, and 500 mg consisted exclusively of male participants ($n=3$ per cohort). The 250 mg cohort included two males and one female, and the largest cohort, receiving the highest dose of 625 mg ($n=8$), included seven males and one female.

The mean age of patients across all cohorts was comparable, with no statistically significant differences between groups ($p=0.279$). The youngest participants were in the second cohort

(250 mg; mean age, 59.0 ± 11.3 years), and the oldest were in the fifth cohort (625 mg; mean age, 70.2 ± 5.9 years). The overall mean age for the study population was 65.8 ± 9.6 years.

Body mass index (BMI) was also comparable among groups. The highest mean BMI was observed in the second cohort (32.0 ± 7.0 kg/m²), and the lowest in the fourth cohort (500 mg; 24.3 ± 1.5 kg/m²). The mean BMI for the entire sample was 27.6 ± 5.1 kg/m², which corresponds to the overweight category.

All patients had received at least one prior line of standard systemic therapy before study enrollment, and 60% (12 of 20) had received two or more lines. The most common class of prior therapy was androgen deprivation therapy (85%), reflecting the high proportion of patients with prostate cancer ([Table 1](#)).

On the ECOG performance status scale, most patients had a score of 1 ($n=12$) or 2 ($n=8$) at screening. The proportion of patients with ECOG=2 was higher in cohort 5 (six of eight) than in the other cohorts, each of which had \leq one of three patients with ECOG=2; however, this difference was not statistically significant (χ^2 test: $p=0.085$). Thus, all dose groups were comparable in terms of key baseline characteristics ([Table 2](#)).

Table 1. Baseline demographic and clinical characteristics by dose cohort

Cohort (dose)	Main tumor types (n)	Mean prior treatment lines (range)	Major drug classes received (cohort frequency)
1 (125 mg)	Prostate cancer (2), bladder cancer (1)	2 (1-3)	Chemotherapy (platinum-based) – 67%; androgen-deprivation therapy – 100%; immunotherapy – 33%
2 (250 mg)	Prostate cancer (1), kidney cancer (1), bladder cancer (1)	3 (2-4)	Chemotherapy – 100%; androgen-deprivation therapy – 33%; targeted therapy – 67%; immunotherapy – 33%
3 (375 mg)	Prostate cancer (2), bladder cancer (1)	2 (1-3)	Chemotherapy – 100%; androgen-deprivation therapy – 100%
4 (500 mg)	Prostate cancer (3)	1 (1-2)	Androgen-deprivation therapy – 100%
5 (625 mg)	Prostate cancer (5), kidney cancer (1), bladder cancer (1), penile cancer (1)	3 (2-5)	Chemotherapy – 63%; androgen-deprivation therapy – 100%; targeted therapy – 38%; immunotherapy – 38%; radiotherapy – 25%
Total	Prostate cancer (13), kidney cancer (2), bladder cancer (4), other (1)	2.4 (1-5)	Androgen-deprivation therapy – 85%; chemotherapy – 60%; targeted therapy – 35%; immunotherapy – 25%; radiotherapy – 10%

Targeted therapy includes small-molecule or antibody-based targeted agents; immunotherapy refers to immune-checkpoint inhibitors. Frequencies are reported within each cohort.

Table 2. Baseline characteristics by dose cohort

Cohort (dose)	n	Gender (M/F)	Age, years (mean \pm SD)	Weight, kg (mean \pm SD)	BMI, kg/m ² (mean \pm SD)	ECOG 1/2, n
1 (125 mg)	3	3/0	63.0 \pm 2.6	77.6 \pm 11.6	26.8 \pm 4.6	3/0
2 (250 mg)	3	2/1	59.0 \pm 11.3	90.0 \pm 21.0	32.0 \pm 7.0	2/1
3 (375 mg)	3	3/0	69.7 \pm 19.1	72.3 \pm 3.2	26.7 \pm 0.6	2/1
4 (500 mg)	3	3/0	59.7 \pm 4.0	76.7 \pm 14.6	24.3 \pm 1.5	3/0
5 (625 mg)	8	7/1	70.2 \pm 5.9	78.6 \pm 14.7	27.9 \pm 5.9	2/6
Total	20	18/2	65.8 \pm 9.6	78.9 \pm 13.8	27.6 \pm 5.1	12/8

Table 3. Vital signs before and 28 days after a single dose of AV-22 (n=20)

Parameter (unit)	Pre-dose (mean \pm SD)	Day 28 (mean \pm SD)	Change (mean \pm SD)
Axillary temperature (°C)	36.58 \pm 0.10	36.54 \pm 0.08	-0.04 \pm 0.15
Systolic BP (mmHg)	137.35 \pm 7.10	136.10 \pm 5.40	-1.25 \pm 4.96
Diastolic BP (mmHg)	84.95 \pm 8.00	85.60 \pm 6.81	0.65 \pm 5.36
Heart rate (beats/min)	76.95 \pm 12.39	73.05 \pm 9.23	-3.90 \pm 11.37
Respiratory rate (breaths/min)	18.55 \pm 1.36	17.45 \pm 1.10	-1.10 \pm 1.02

Table 4. Hematological parameters before and 28 days after a single dose of AV-22 (n=20)

Parameter (unit)	Pre-dose (mean±SD)	Day 28 (mean±SD)	Change (mean±SD)
Hemoglobin (g/L)	127.90±14.65	126.50±15.94	-1.40±10.05
Leukocytes (× 10 ⁹ /L)	7.03±1.69	7.66±2.37	0.63±1.80
Neutrophils (× 10 ⁹ /L)	4.58±1.50	4.67±2.00	0.09±1.65
Platelets, (10 ⁹ /L)	255.75±73.12	268.55±84.48	12.80±53.98

Table 5. Biochemical parameters of liver and renal function before and 28 days after a single dose of AV-22 (n=20)

Parameter (unit)	Pre-dose (mean±SD)	Day 28 (mean±SD)	Change (mean±SD)
ALT (U/L)	18.34±8.60	20.43±10.09	2.09±10.13
AST (U/L)	22.68±7.02	23.82±6.53	1.14±7.07
Total bilirubin (μmol/L)	14.95±9.95	12.11±6.83	-2.84±5.19
Creatinine (μmol/L)	89.22±20.27	94.35±16.99	5.13±8.53
Urea (mmol/L)	5.84±1.54	6.71±1.63	0.87±1.59
ALP (U/L)	83.15±29.81	95.42±24.96	12.27±16.81
CrCl (mL/min/1.73 m ²)	79.28±27.17	74.31±25.78	-4.97±7.82

Table 6. Hemostasis parameters before and 14 days after a single dose of AV-22 (n=20)

Parameter	Pre-dose, Day 1 (mean±SD)	Day 14 (mean±SD)	Change, Day 14-Day 1 (mean±SD)
Prothrombin index (%)	97.81±18.07	96.64±20.08	-1.17±18.01
Prothrombin time (s)	10.96±0.82	10.97±0.81	0.01±0.82
INR	1.04±0.08	1.04±0.08	-0.00±0.08
APTT (s)	29.20±4.22	28.64±2.60	-0.56±4.23
Thrombin time (s)	18.63±2.22	17.88±0.81	-0.75±2.35

Table 7. Electrocardiographic parameters before and 28 days after a single dose of AV-22 (n=20)

Parameter	Pre-dose, Day 1 (mean±SD)	Day 28 (mean±SD)	Change, Day 28-Day 1 (mean±SD)
Heart rate (bpm)	74.85±13.20	73.95±11.06	-0.90±13.61
PR interval (ms)	155.21±27.48	159.89±20.47	4.68±25.32
QRS duration (ms)	64.80±19.86	62.50±17.93	-2.30±23.42
QT interval (ms)	349.40±40.81	354.60±46.38	5.20±44.56
QTc (ms)	388.75±46.98	392.65±47.98	3.90±38.97

Table 8. Results of longitudinal analysis by visit: Friedman test with Benjamini-Hochberg FDR correction

Indicator	Complete cases, n	p (Friedman)	p FDR
ALT (U/L)	20	0.5998	0.5998
Creatinine (μmol/L)	20	0.2033	0.2710
Hemoglobin (g/L)	20	0.0051	0.0136
Pulse (bpm)	20	0.0511	0.1022
QTc (ms)	20	0.3490	0.3989
SBP (mmHg)	20	0.0800	0.1279
Thrombin time (s)	20	0.0010	0.0039
Total bilirubin (μmol/L)	20	0.0002	0.0016

The functional status, as assessed by the ECOG performance status scale, remained stable in all 20 patients throughout the observation period: 12 patients (60%) maintained a score of 1, and 8 patients (40%) maintained a score of 2.

Analysis of vital sign dynamics in the total patient cohort (n=20) following a single dose of the study drug revealed no clinically significant changes over the 28-day observation period. By day 28, a statistically insignificant decrease in mean heart rate (HR) of 3.9±11.37 bpm was observed (from 76.95±12.39 to 73.05±9.23 bpm), along with a reduction in respiratory rate of 1.10±1.02 breaths per minute (from 18.55±1.36 to 17.45±1.10 breaths per minute). Average blood pressure readings remained stable: systolic

blood pressure (SBP) decreased slightly by 1.25±4.96 mmHg (from 137.35±7.10 to 136.10±5.40 mmHg), and diastolic blood pressure (DBP) increased minimally by 0.65±5.36 mmHg (from 84.95±8.00 to 85.60±6.81 mmHg). Axillary body temperature also showed no significant change (-0.04±0.15 °C). Thus, all recorded fluctuations were within physiological norms and lacked clinical significance, confirming the absence of a systemic effect of a single dose of AV-22 on core vital functions (Table 3).

Analysis of key hematological parameters in the overall patient sample (n=20) 28 days after a single dose of AV-22 revealed no evidence of myelosuppression or clinically significant toxic changes in the hematopoietic system. Hemoglobin levels remained stable, showing a minimal decrease of 1.40±10.05 g/L (from 127.90±14.65 to 126.50±15.94 g/L), which falls within the range of physiological variability. White blood cell counts showed no significant alteration: leukocyte count increased by 0.63±1.80×10⁹/L (from 7.03±1.69 to 7.66±2.37×10⁹/L), and absolute neutrophil count remained virtually unchanged (increase of 0.09±1.65×10⁹/L). The observed increase in platelet count of 12.80±53.98×10⁹/L (from 255.75±73.12 to 268.55±84.48×10⁹/L) was also insignificant and consistent with normal biological variability.

Thus, a single dose of the study drug within the investigated dose range had no clinically significant effect on the principal parameters of clinical blood analysis over the 28-day observation

period. All recorded changes were random and remained within reference values ([Table 4](#)).

The dynamics of key biochemical indicators of liver and kidney function 28 days after a single dose of AV-22 demonstrated overall stability, with no evidence of clinically significant hepatotoxicity or nephrotoxicity. Liver cytotoxicity markers remained within normal limits: alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities increased slightly by 2.09 ± 10.13 U/L and 1.14 ± 7.07 U/L, respectively. In contrast, total bilirubin levels decreased by 2.84 ± 5.19 $\mu\text{mol/L}$. The most notable change was observed for alkaline phosphatase (ALP), whose activity increased by an average of 12.27 ± 16.81 U/L; however, this change also remained within physiological limits. Renal function parameters showed minimal shifts: serum creatinine levels increased by 5.13 ± 8.53 $\mu\text{mol/L}$, and urea levels by 0.87 ± 1.59 mmol/L. Concurrently, the estimated glomerular filtration rate (eGFR) decreased slightly by 4.97 ± 7.82 mL/min/1.73 m². These changes are not clinically significant and reflect natural variability in the measured parameters. Thus, a single dose of AV-22 had no adverse effect on liver or kidney function in patients over the 28-day observation period ([Table 5](#)).

Assessment of hemostasis parameters 14 days after a single dose of AV-22 revealed no clinically significant deviations, indicating that the drug has no effect on the blood coagulation system. All key coagulation parameters remained stable. The prothrombin index (PTI) and prothrombin time (PT) remained virtually unchanged, with fluctuations of $\pm 1.17\%$ and ± 0.01 s, respectively. The international normalized ratio (INR) remained unchanged. The activity of the intrinsic coagulation pathway, as assessed by activated partial thromboplastin time (APTT), decreased by 0.56 ± 4.23 s, and thrombin time – which characterizes the final stage of clot formation – decreased by 0.75 ± 2.35 s. These minimal changes in all parameters fall within normal laboratory and physiological variability and have no clinical significance. Thus, a single dose of the study drug AV-22 has no statistically or clinically significant effect on the state of the blood coagulation system over a two-week observation period ([Table 6](#)).

Analysis of electrocardiogram parameters 28 days after a single dose of AV-22 in all 20 patients revealed no clinically significant changes indicative of a negative effect of the drug on cardiac electrophysiology. Heart rate and conduction parameters remained stable. The average heart rate (HR) remained virtually unchanged (decrease of 0.90 ± 13.61 beats per minute). Minor fluctuations were observed in the durations of the intervals: the PR interval increased by 4.68 ± 25.32 ms, the QRS complex duration decreased by 2.30 ± 23.42 ms, and the QT interval increased by 5.20 ± 44.56 ms. The key safety parameter, the corrected QT interval (QTc), did not show a clinically significant increase, changing on average by only 3.90 ± 38.97 ms (from 388.75 ± 46.98 ms to 392.65 ± 47.98 ms) (Friedman's criterion, $p=0.349$).

Thus, a single dose of AV-22 did not cause any significant changes in ECG parameters over the 28-day observation period, which is indicative of no proarrhythmic potential and no effect on cardiac conduction within the studied dose range ([Table 7](#)).

A longitudinal design was employed to assess the dynamics of safety parameters across scheduled visits. Following identification of the overall trend using the Friedman test, post-hoc paired comparisons were conducted according to a “screening versus each subsequent visit” framework to identify specific time points with

significant changes. All pairwise comparisons were performed using the nonparametric Wilcoxon signed-rank test for related samples. To control for multiple comparisons, the Benjamini–Hochberg procedure was applied to adjust the false discovery rate (FDR) ([Tables 8](#) and [9](#)).

Statistically significant differences ($p\text{FDR} < 0.05$) relative to baseline (screening) after FDR correction were observed for only a limited number of parameters, underscoring the overall stability of most assessed variables. Hemoglobin levels decreased significantly but reversibly on day 1 after drug administration (median change: -7 g/L; $p < 0.01$), with a tendency toward recovery by day 7 and complete normalization by day 28. A moderate, statistically significant increase in total bilirubin was observed on day 1 (median: $+2.0$ $\mu\text{mol/L}$; $p < 0.01$) and day 7 (median: $+1.8$ $\mu\text{mol/L}$; $p < 0.05$), with return to baseline levels by days 14 and 28.

Concurrently, markers of liver function (ALT, AST, ALP) and kidney function (creatinine) remained stable throughout the observation period. In the hemostasis system, a short-term insignificant increase in thrombin time was noted only on day 1 (median: $+0.9$ s; $p < 0.01$). In no cohort did any parameter reach a grade of ≥ 3 according to CTCAE v4.03.

Parameters with borderline or no significance ($p\text{FDR} > 0.05$)

The integral cardiovascular safety parameter, the QTc interval, demonstrated no statistically significant temporal variation across visits ($p=0.349$). No critical absolute QTc prolongation (>480 ms) was recorded. However, in seven of twenty patients, an increase in QTc relative to screening values exceeding 30 ms was observed; in four of twenty, the increase exceeded 60 ms – findings likely attributable to baseline variability. Other vital parameters remained stable: systolic blood pressure and heart rate showed no significant alterations following adjustment for multiple comparisons.

Descriptive analysis of the occurrence of adverse events and laboratory abnormalities did not reveal a clear dose-dependent pattern. The most frequently reported transient abnormalities – including decreased hemoglobin and fluctuations in bilirubin – occurred with comparable frequency across all cohorts, including the lowest dose group.

Correlation analyses, which assessed relationships between baseline screening parameters and their changes at day 28 ($\Delta\text{Day 28}$), revealed several statistically significant associations following adjustment for multiple comparisons (FDR). These relationships were largely consistent with expected physiological coupling of parameters. Virtually complete agreement was confirmed between changes in pulse rate and heart rate as measured by ECG ($\rho=0.988$, $q=1.21 \times 10^{-12}$), as well as a strong association between red blood parameters: hemoglobin level and red blood cell count ($\rho=0.926$, $q=4.72 \times 10^{-6}$). A strong inverse correlation was observed between serum creatinine levels and estimated glomerular filtration rate ($\rho=-0.839$, $q=0.00142$). However, no dose-dependent relationship was detected between the administered dose of AV-22 and change in QTc interval (ΔQTc ; $\rho=-0.496$, $q=0.348$). Overall, the significant correlations reflected established physiological and mathematical relationships (e.g., between prothrombin time and INR) and did not suggest a novel or compelling pattern of organ-specific toxicity. Thus, these findings are purely exploratory and hypothesis-generating, intended to inform the design of future studies.

Table 9. Post-hoc pairwise comparisons with the screening visit (Wilcoxon matched-pairs signed-rank test with Benjamini-Hochberg FDR correction) for each parameter

Indicator (units)	Comparison (with screening)	Sample size (n)	Median change [Q1; Q3]	Wilcoxon p	Benjamini-Hochberg q
Hemoglobin (g/L)	Screening vs. Day 1 (+1 h)	20	-7.0 [-10.2; -3.0]	0.0009	0.0060
Hemoglobin (g/L)	Screening vs. Day 2	20	-5.5 [-7.5; 0.2]	0.0042	0.0099
Hemoglobin (g/L)	Screening vs. Day 7	20	-5.5 [-9.0; -3.0]	0.0025	0.0088
Hemoglobin (g/L)	Screening vs. Day 14	2	-4.5 [-10.2; 2.0]	0.0267	0.0468
Thrombin time (s)	Screening vs. Day 1 (+1 h)	20	0.9 [0.3; 1.6]	0.0003	0.0020
Total bilirubin (μmol/L)	Screening vs. Day 1 (before admission)	20	1.80 [0.15; 5.43]	0.0058	0.0136
Total bilirubin (μmol/L)	Screening vs. Day 1 (+1 h)	20	1.45 [-0.18; 3.23]	0.0328	0.0459
Total bilirubin (μmol/L)	Screening vs. Day 2	20	2.25 [0.40; 5.55]	0.0049	0.0136
Total bilirubin (μmol/L)	Screening vs. Day 3	20	1.65 [0.28; 4.83]	0.0023	0.0136
Total bilirubin (μmol/L)	Screening vs. Day 7	20	2.73 [-0.60; 4.17]	0.0266	0.0459

Only comparisons with $q < 0.05$ are presented.

Table 10. Serum tumor-marker dynamics (n=20)

Marker (Units)	Baseline Point	N	Friedman Test (χ^2 , p)	Descriptive Statistics (Median [Q1; Q3]) by Visit
β-hCG (mIU/mL)	Screening	20	$\chi^2 = 7.31$; p = 0.063	Scr: 0.50 [0.50; 1.11]
				D3: 0.51 [0.50; 0.99]
				D7: 0.50 [0.50; 1.03]
				D28: 0.52 [0.50; 1.03]
AFP (IU/mL)	Screening	20	$\chi^2 = 2.83$; p = 0.434	Scr: 2.11 [1.70; 2.79]
				D3: 2.19 [1.57; 3.15]
				D7: 2.03 [1.73; 2.60]
				D28: 2.15 [1.68; 2.86]
PSA total (ng/mL)	Day 1	18	$\chi^2 = 3.98$; p = 0.266	D1: 1.44 [0.95; 3.17]
				D3: 1.40 [0.86; 2.84]
				D7: 1.46 [0.97; 3.82]
				D28: 1.41 [1.06; 2.97]
PSA free (ng/mL)	Day 1	18	$\chi^2 = 14.85$; p = 0.0017*	D1: 0.39 [0.29; 0.67]
				D3: 0.36 [0.24; 0.62]
				D7: 0.43 [0.25; 0.63]
				D28: 0.41 [0.30; 0.64]
% PSA free	Day 1	18	$\chi^2 = 0.18$; p = 0.983	D1: 23.5 % [18.3 %; 42.0 %]
				D3: 21.5 % [14.5 %; 43.0 %]
				D7: 24.0 % [17.0 %; 45.0 %]
				D28: 28.0 % [18.3 %; 43.0 %]

Scr, screening; D1/3/7/28, Day 1/3/7/28. For total and free PSA, n=18 (males only). *Statistical significance did not persist after correction for pairwise comparisons.

Pharmacokinetic analysis

In all selected plasma samples from 20 patients receiving various doses of AV-22, concentrations of the target analytes (verbascoside, cucurbitacin D, cucurbitacin E, and cucurbitacin I) were below the established lower limit of quantification (1 ng/mL). Consequently, pharmacokinetic parameters – including AUC, C_{max} , T_{max} , $T_{1/2}$, and CL – could not be calculated. This outcome is consistent with observations reported for many herbal medicinal products, as documented in the official prescribing information of established products such as Kanefron®, Sinupret®, plantain syrup, and Hofitol®.

In this Phase I study, evaluation of antitumor activity was neither a primary nor a secondary objective; the protocol was designed to assess safety and tolerability. Among the 20 patients who completed the 28-day observation period, follow-up imaging (ultrasound, MRI) showed no clinically evident rapid disease progression requiring urgent therapeutic intervention. Imaging performed during standard follow-up revealed no new safety

signals related to tumor growth. Nevertheless, the safety-driven design and brief follow-up preclude interpreting stability indicators as evidence of antitumor efficacy for the investigational botanical extract AV-22; such conclusions require controlled phase II studies with longer observation windows.

As part of the study to assess the short-term impact of therapy, serum tumor-marker dynamics (β-hCG, AFP, total and free PSA) were analyzed in 20 patients at screening and on days 1, 3, 7, and 28 (Table 10). Owing to differences in data availability, screening served as the time reference point for β-hCG and AFP (n=20), whereas day 1 served as the time reference point for PSA (n=18, males only). Statistical analysis comprised descriptive statistics (mean±SD, median [Q1, Q3]) and the Friedman non-parametric test for repeated measurements, followed by pairwise comparisons using Wilcoxon signed-rank tests with Holm's correction. Short-term dynamics of the markers showed no statistically significant, homogeneous alteration in the patient group over 28 days. However, the analysis revealed marked inter-individual variability

determined by tumor biology rather than the overall therapeutic effect.

The β -hCG level remained stable across the group ($p=0.063$); the median at every visit was 0.50 mIU/mL, a value that likely reflects the assay's lower limit of detection. The group trend was driven by two patients (10%) who reached ≥ 5 mIU/mL on day 28, an increase of up to +6.14 units from screening. Two additional patients showed an increase ≥ 1.0 mIU/mL. These findings may reflect ectopic marker production or tumor heterogeneity rather than a drug-induced shift over 28 days.

AFP concentration likewise showed no significant change ($p=0.434$). Values in all patients remained within a narrow reference range (median ≈ 2.1 - 2.2 IU/mL), and the largest individual rise by day 28 did not exceed 1.46 units. This stability is clinically expected because AFP is not a primary marker for most genitourinary neoplasms (except germ-cell tumors), and synchronous short-term fluctuations during therapy are unlikely.

Among the 18 men, total PSA levels revealed no uniform 'PSA response.' Total-PSA dynamics were statistically insignificant ($p=0.266$); the median relative change from day 1 to day 28 was +3.9%, with a broad interquartile range of -10.7% to +21.2%. At the same time, individual trajectories showed marked dispersion: three patients (16.7%) exhibited an increase of $\geq 50\%$, and one patient (5.6%) exhibited a decrease of $\geq 50\%$. Free PSA displayed a weak statistical signal ($p=0.0017$), yet none of the pairwise comparisons reached significance after correction. A non-specific pattern was observed – a decrease by day 3 in 14 of 18 patients, followed by a return to baseline or above baseline by day 28. The proportion of free PSA (%) remained absolutely stable ($p=0.983$).

The data obtained indicate the absence of a synchronous, statistically significant effect of short-term (28-day) therapy on the levels of the tumor markers studied in the group as a whole. The observed variability – especially for PSA and β -hCG – likely reflects individual characteristics of tumor biology, population heterogeneity, and analytical variation rather than a pharmacodynamic effect. Accurate assessment of a potential marker response requires identification of homogeneous nosological subgroups (e.g., prostate cancer patients for PSA) and a substantially longer observation period (≥ 12 weeks), together with imaging data and clinical evaluation.

Maximum tolerated dose

The study demonstrated that single oral doses of 125-625 mg of AV-22 were safe and well tolerated; the planned maximum of 625 mg was attained. Dose-limiting toxicity was not observed at any of the five dose levels, precluding classic MTD determination. Escalation beyond 625 mg, necessary for formal MTD assignment, was not stipulated in the study protocol. Consequently, AV-22 exhibited a favorable tolerability profile up to the highest dose tested (625 mg), supporting this dose as a safe starting point for subsequent studies with a repeat-dosing regimen.

Discussion

This study represents the first clinical evaluation of the safety of AV-22, a standardized extract of *Gratiola officinalis* L., in patients with advanced genitourinary cancers. The key finding is the absence of DLTs and serious adverse events across single doses of 125 to 625 mg, confirming the favorable tolerability profile observed in

preclinical studies. Minor and transient changes in laboratory parameters – including modest decreases in hemoglobin and fluctuations in bilirubin – were not associated with clinical consequences and are consistent with effects commonly observed with other cytotoxic agents. Notably, no dose-dependent QTc prolongation or signs of cardiotoxicity were detected.

Despite the overall favorable profile, the statistically significant laboratory shifts identified warrant further investigation. The transient reduction in hemoglobin observed on day 1 may indicate hemodilution or a minimal effect on erythropoiesis; however, this change had no clinical consequences following single-dose administration. A short-term elevation in total bilirubin levels, with stable transaminase levels, may indicate mild, reversible inhibition of transport proteins (e.g., OATP1B1/B3) or competition for the glucuronidation pathway, a phenomenon characteristic of certain plant-derived polyphenols [18-20]. However, the present study does not provide direct mechanistic evidence for these effects in the context of AV-22, and further investigation is warranted, particularly in patients with pre-existing liver dysfunction or anemia and in the setting of repeated dosing.

As the maximum tolerated dose (MTD) was not reached, the single-dose therapeutic window of AV-22 appears wide. Preclinical studies of *Gratiola officinalis* L. extracts have demonstrated antitumor effects in various models and reported apoptosis-inducing activity in human tumor cell cultures [9, 10], as well as potential technological approaches to modifying the dosage form (e.g., microencapsulation) [11]. However, the safety and tolerability of plant extracts cannot be extrapolated from preclinical data without proper clinical verification, especially because some classes of plant triterpenoids – including cucurbitacins – are characterized by a narrow therapeutic window and non-specific toxicity [7]. From this perspective, the results obtained are a necessary step in translation and create the basis for subsequent phase Ib/IIa clinical trials with multiple doses of AV-22. Imaging-based assessment of tumor response according to Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 at day 28 revealed no progression in the included patients. However, the design and duration of observation in this study are safety-oriented and do not allow the interpretation of stability indicators as evidence of the antitumor efficacy of the investigational drug; such conclusions require controlled Phase II studies with adequate time horizons for evaluation.

Limitations

As with any Phase I study, methodological constraints must be acknowledged when interpreting the results. The trial generated data solely on the safety and tolerability of a single dose of the investigational drug. The open-label, non-randomized, single-center design may introduce subjective systematic error (bias). The relatively small sample size ($n=20$), dictated by the 3+3 dose-escalation design, limits detection of rare adverse events. Consequently, among patients receiving the highest dose (625 mg), the proportion with the poorest functional status (ECOG 2) was higher than in other groups; although this difference did not achieve formal statistical significance, possibly due to the small sample size, such an imbalance could potentially affect the overall tolerability assessment at this dose level.

Including patients with heterogeneous genitourinary neoplasm nosologies, though justified for initial safety assessment, precludes

extrapolation of findings to individual cancer types without additional studies.

Although pharmacokinetic sampling was performed, verbasco-side and cucurbitacins D/E/I concentrations in all plasma samples were below the limit of quantitation, preventing calculation of pharmacokinetic parameters and restricting interpretation of exposure and potential dose-dependent effects. The assertion of a “wide therapeutic window,” based only on the absence of dose-limiting toxicities across the tested dose range, is preliminary. Without plasma concentration data, systemic exposure, pharmacokinetic linearity, and potential accumulation cannot be assessed – information essential for defining a multiple-dose regimen. Acquisition of robust pharmacokinetic data should be mandatory in subsequent development phases.

Safety data must remain distinct from efficacy inferences. Although no overt rapid progression was observed in any patients at the 28-day follow-up, this data – attributable to the single-dose, short-term design – does not constitute evidence that AV-22 possesses antitumor activity. Determination of pharmacokinetic parameters, antitumor efficacy, and the safety profile of repeated dosing will require future investigations. These limitations are inherent to first-in-human pilot trials and delineate the objectives for subsequent, more definitive clinical development.

Conclusion

A single 125-625 mg dose of AV-22 in patients with advanced genitourinary malignancies was initially safe and well tolerated. No dose-limiting toxicities were observed at any of the five tested levels, precluding identification of a conventional maximum tolerated dose. The 625 mg dose, being the highest tested, can be considered safe for initiating studies with repeated administration. These findings support the feasibility of further investigations involving multiple administrations of AV-22, expanded biomarker analyses, and standardized clinical efficacy criteria.

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Conflicts of interest

The authors declare that they have no conflict of interest.

Authors' Contributions

R.N. Fomkin conceptualized the study; framed the theoretical basis, hypothesis, aims, and objectives; designed the protocol and defined patient eligibility criteria; developed and validated the core methodologies; interpreted the findings and verified their scientific context and relevance; and drafted and finalized the manuscript.

T.Yu. Kalyuta conducted the systematic literature review, interpreted the data within the methodological framework, performed technical editing, compiled the bibliography, and facilitated team communication.

A.S. Fedonnikov reviewed pertinent publications, executed the patent landscape search, and compared the study results with existing analogues.

V.M. Popkov formulated the overarching scientific concept, developed theoretical models, provided scientific supervision, coordinated the research team, and verified and critically evaluated the final results.

O.A. Fomkina reviewed pertinent literature and patents; processed primary documentation and the electronic database; created and described tables and key visualizations; performed statistical analyses; interpreted and reported statistical significance; edited the manuscript for scientific accuracy; compared the findings with published work; and drafted the Discussion section.

D.A. Durnov managed enrolled patients; prepared primary documentation and informed consent forms; collected biological specimens; and participated in the clinical interpretation of the data.

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