Questionnaires (open from 7th of April to 7th of June 2021)

I. Introduction

The purpose of the questionnaires was to further assess the clinical, patient and technical needs, as well as the readiness of the solution providers to address the users' needs. The project consortium took all the defined needs and requirements from the Virtual OMC Event held in March into account to define the questions included in questionnaires addressed at different stakeholders.

The questionnaires were designed to obtain broader and quantitative insights from different stakeholder groups – users, solution providers and patient associations (Table 1) – and made publically available on the project website. All the answers were then considered for the definition of the final specifications and evaluation criteria.

Table 1. Number of responses received for the three questionnaires. The total number of responses is depicted in the second column. The number of responses to the individual sections of the questionnaire is shown in the remaining columns.

	Total	Clinical Needs	Childhood Cancer Patients	Adult Cancer Patients	Pre-analytics and library preparation (Lot 1)	Sequencing (Lot 2)	Bioinformatics (Lot 3)	Reporting (Lot 4)
Users	**18**	16	-	-	7	1	3	11
Solution Providers	**48**	-	-	-	22	12	23	18
Patient Associations	**40**	-	27	25	-	-	-	-

Main lessons learned

II. Clinical Needs questionnaire

- We received responses from users predominantly representing hospitals and diagnostic laboratories, 75% of which treat childhood cancers and 56% adult cancers.

The users' answers give us a good overview on the major issues that need to be overcome by the innovative solutions covering the different lots:

- Importance of short turn-around time

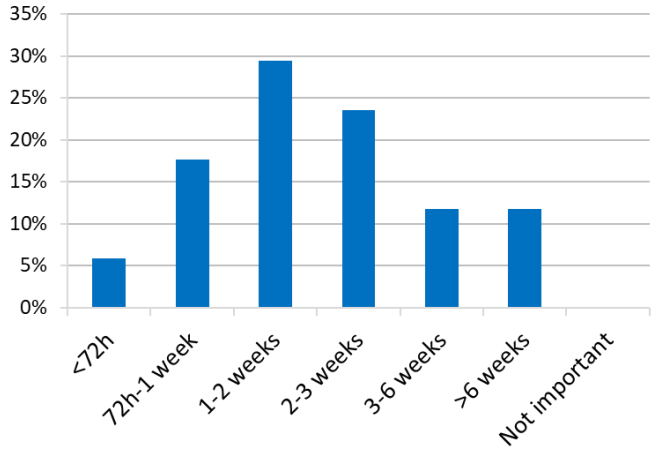


Figure 1. The desired turn-around time by users, from sample collection to NGS reporting

- Information to be covered by NGS

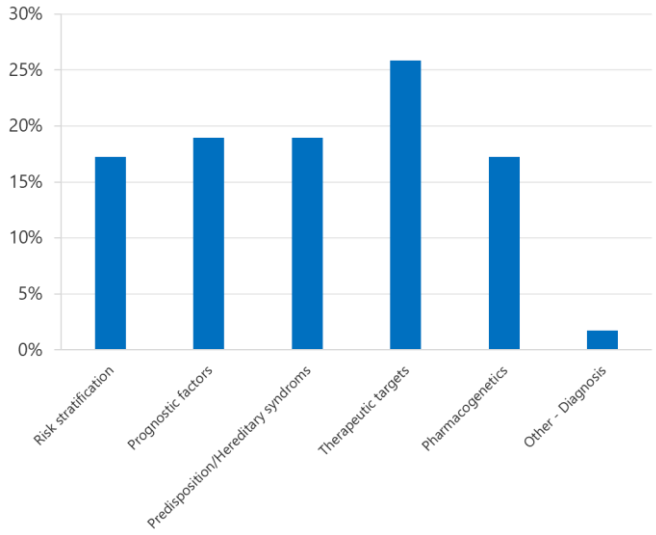


Figure 2. The desired information to be covered by the NGS workflow

- Compatibility of the NGS workflow with different starting material types (including liquid biopsies)

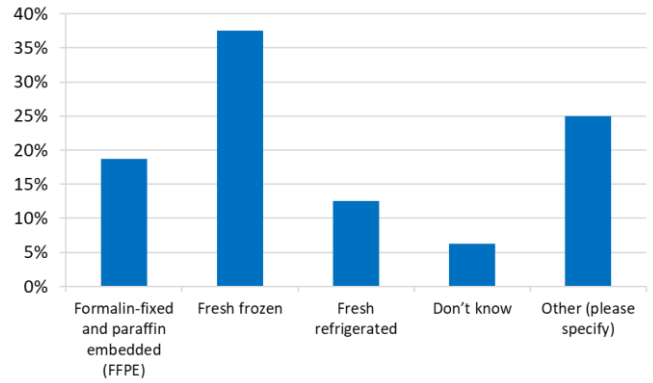


Figure 3. The desired compatibility of the workflow with different starting materials

- Types of genetic variants relevant for cancer predisposition: **SNV/small indels, CNV, fusions**
- Types of genetic variants relevant as actionable items: **fusions, SNV/small indels, overexpression, CNV**

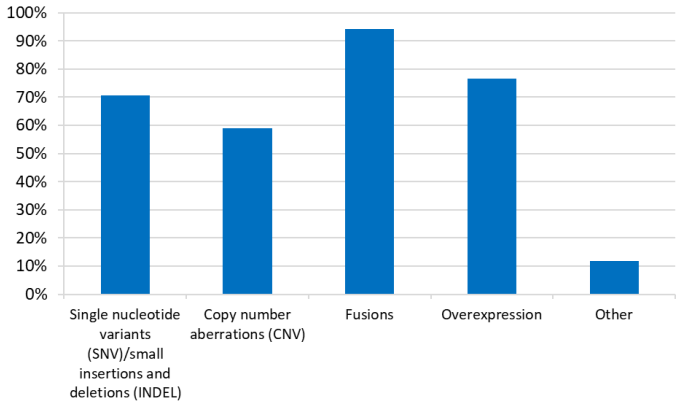
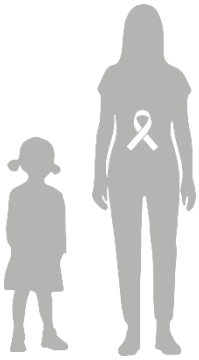


Figure 4. Relevance of types of genetic variants as actionable items

- Inclusion of a variety of genes and genetic variant types into the NGS workflow for cancer diagnosis and therapy decision-making, which will have implications in the choice of NGS technology
- Comprehensive reporting, including:
    - level of evidence of genetic variants for suggested treatments, dosing and treatment schedules.
    - response predictions to targeted therapies, pharmacogenetic information, suspected germline mutations, suspected clonal hematopoiesis and possibilities to match genetic findings to active clinical trials.



III. Patients' Needs questionnaire

We were able to assess the current level of knowledge on NGS and what advantages it has, but also which concerns might arise among patients and what are their current unmet needs.

- A total of 40 organizations from 24 countries responded
  - 27 organizations representing childhood cancer patients,
  - 25 organizations representing adult cancer patients
- Limited knowledge and experience of organizations with NGS, leading to insufficient preparation to advise patients
- Poor knowledge among patients/parents about the possibility of using NGS in cancer diagnosis and therapy decision-making

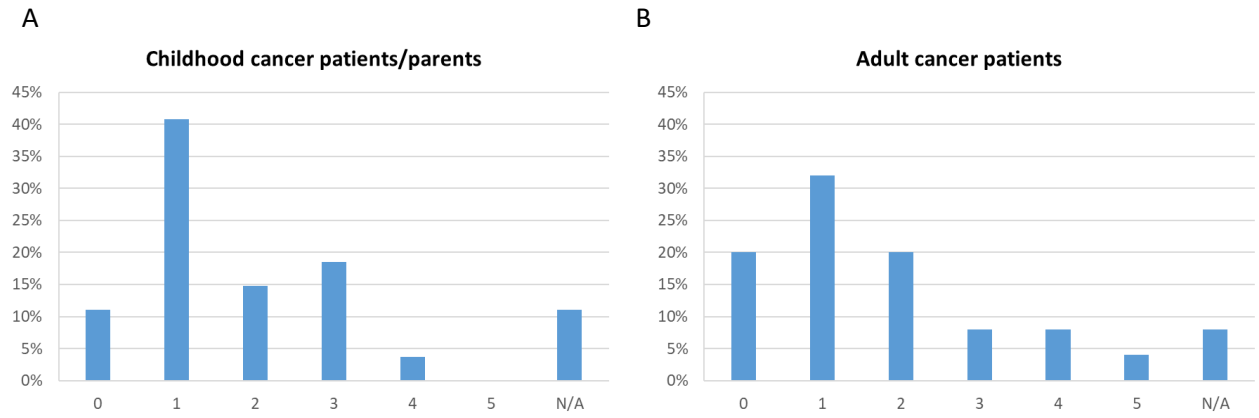


Figure 5. Knowledge of patients about the use of NGS in cancer diagnostics, according to the patient associations. A. Knowledge of childhood cancer patients and parents; B. Knowledge of adult cancer patients. Scoring scale: 0-5; 0 = no knowledge at all; 5 = very high knowledge; N/A = not applicable.

In summary, the patient needs questionnaire has revealed the low level of knowledge about the use of NGS in cancer diagnosis among patients and the importance of providing patients with clear information:

Before testing, patients consider very important to receive comprehensive information about the diagnostic procedure and the evidence for basing treatment decision on NGS

- which NGS data will be used and its purpose, advantages and risks of NGS, impact on their lives and the lives of their families, how data security will be guaranteed

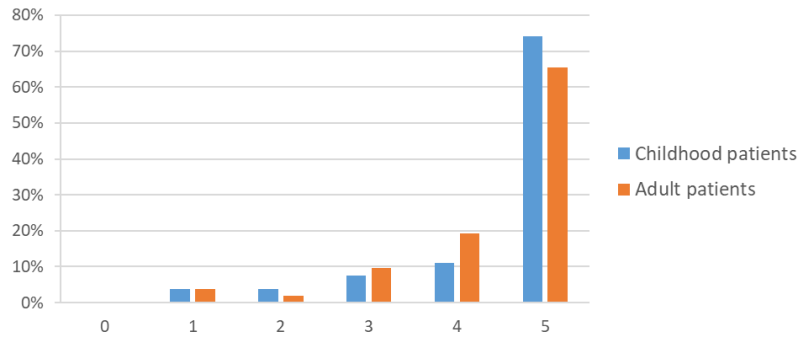


Figure 6. Importance given by adult and childhood cancer patients to receiving information about the diagnostic procedure before sequencing. Scoring scale: 0-5; 0 = not important; 5 = very important; N/A = not applicable.

- After testing, patients consider very important
  - to receive a report with the results (including the genetic and pharmacogenetic results) and implications on family members
  - to receive support of genetic counseling expert
  - the establishment of a data security level similar to e-banking

Particularly for childhood patients, the answers revealed the importance to:

- assess needs and rights after turning 18 years old
  - important to automatically inform patients about previously performed NGS, ideally in a face-to-face meeting with a well-informed physician, geneticist and psychologist
- receive information about the rights to withdraw data based on GDPR

IV. Technical questionnaires

The answers confirmed the need to develop NGS solutions that cover different sequencing approaches, as previously identified by the Buyers group. This reassures us that the scope of the project is aligned with the current users' interests and with the providers' readiness, including the inclusion of genome-wide approaches.

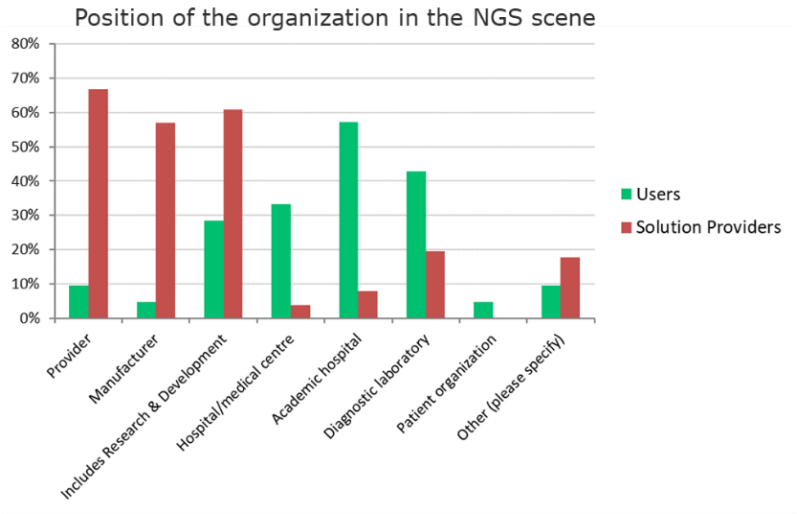


Figure 7. Profile of the responders to the questionnaire, in relation to the A. belonging sector, B. sequencing throughput.

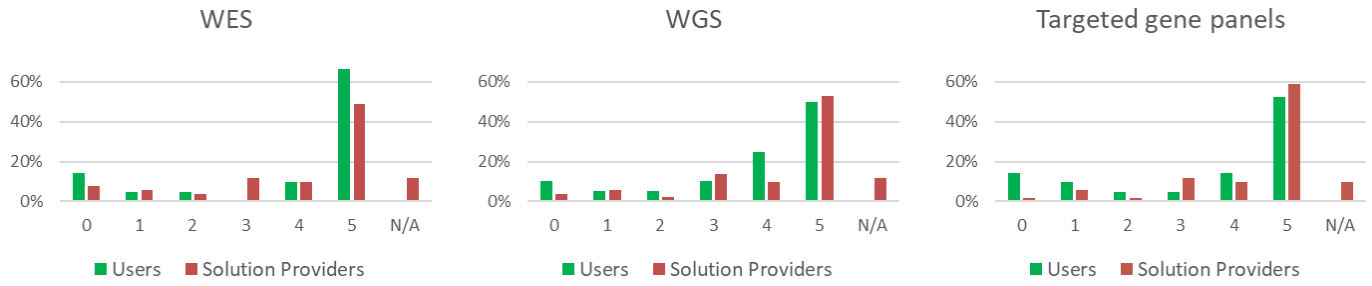


Figure 8. Interest of users and solution providers in whole exome sequencing (WES), whole genome sequencing (WGS) and targeted gene panels. Scoring scale: 0-5; 0 = not interested; 5 = highly interested; N/A = not applicable.

We hereby summarize the main outcomes of the answers to the questions referring to the technical lots of the Instand-NGS4P workflow, which were considered to specify the challenges and evaluation criteria to be addressed by potential solution providers of the PCP.

a) Lot 1 – Pre-analytics and library preparation

- Most commonly used samples for NGS: blood, frozen tissue and FFPE
- Most challenging samples to extract nucleic acids from:
    - DNA and RNA from FFPE and extracellular vesicles,
    - RNA from frozen tissue,
    - cfDNA and cfRNA from blood/plasma

- Most organizations are aware of the different ISO standards, not many work according to them
- Importance of an entry-level quality check of the sample (more important for providers than users)
- Improve library conversion rates and reliability
- Very strong interest in replacing target gene panels with WGS or WES to overcome complications in library preparation related to constantly changing gene lists
- Interest in including pharmacogenomic variants in panels is higher among users than among providers
- Reducing turn-around time:
    - Importance of automation, faster library preparation procedures and integration of multiple steps in library preparation are important for users and providers
- Performance testing, external quality assessment and reference material

Table 2 depicts a ranked list of the challenges in sample and library preparation for users and solution providers.

Table 2. Ranking of the most important challenges in sample and library preparation to overcome in the next 2-3 years. The weighted average score is displayed for users and solution providers. Scoring scale: 0-5; 0 = not important; 5 = highly important.

	USERS	SOLUTION PROVIDERS
Ranking	Challenge	Challenge
1	Compatibility of sample stabilizers with analytical test procedure that need to be performed or have been performed on the same sample (e.g. histology/cytology)	Standardization
2	Standardization	Increase in sample stability
3	Efficiency of the analyte isolation/extraction procedure	Compatibility of sample stabilizers with analytical test procedure that need to be performed or have been performed on the same sample (e.g. histology/cytology)
4	Quality assessment of the isolated analyte	Efficiency of the analyte isolation/extraction procedure
5	Alternative innovative stabilization methods	Automation of the isolation procedures
6	Increase in sample stability	Alternative innovative stabilization methods
7	Increase in sample quantity	Quality assessment of the isolated analyte
8	Increase target concentration in the sample	Increase target concentration in the sample
9	Direct or long range sequencing	Sample storage
10	Automation of the isolation procedures	Direct or long range sequencing
11	Sample storage	Increase in sample quantity
12	Analyte storage	Analyte storage

Table 3 depicts a ranked list of the challenges to decrease the complexity of the complete library preparation procedure for users and solution providers.

Table 3. Ranking of the most important challenges to overcome for reducing the complexity of the library preparation. The weighted average score is displayed for users and solution providers. Scoring scale: 0-5; 0 = not important; 5 = highly important.

	USERS	SOLUTION PROVIDERS
Ranking	Challenge	Challenge
1	Universal approach for different sample types and targets	UMIs mandatory (for mutation detection down to 0.01-0.1% allele frequency)
2	UMIs mandatory (for mutation detection down to 0.01-0.1% allele frequency)	Automation of library preparation
3	Improvement of library preparation success rate	Improvement of library preparation success rate
4	Automation of library preparation	Reduced number of steps needed
5	Automation from nucleic acid isolation (different targets and DNA and RNA, low and high yield) to sequencing (closed system)	Automation of nucleic acid isolation alone
6	Minimal quality and quantity requirements for the input material	Minimal quality and quantity requirements for the input material
7	Improvement of library yield	Universal approach for different sample types and targets
8	Direct or long range sequencing	Improvement of library yield
9	Reduced number of steps needed	Automation from nucleic acid isolation (different targets and DNA and RNA, low and high yield) to sequencing (closed system)
10	Automation of nucleic acid isolation alone	Direct or long range sequencing

b) Lot 2 – Sequencing

Unfortunately, the low number of responses from users to the Lot 2 questions does not allow a direct comparison between existing needs and readiness of solution providers to address them. Nevertheless, the responses from the solution providers give us valuable insights to define Lot 2 specifications.

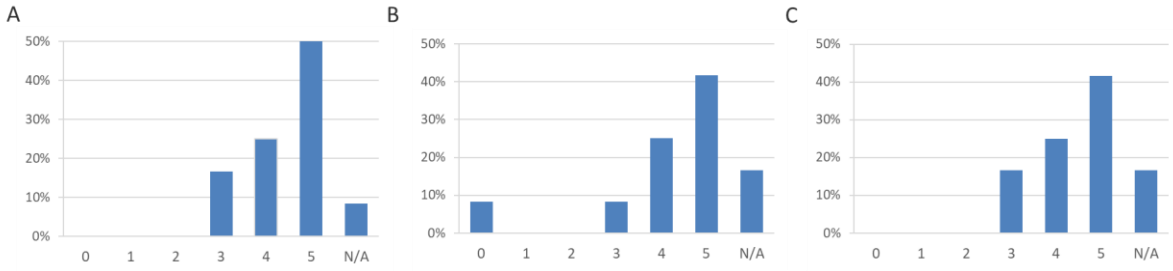


Figure 9. Importance of A) sequencing time, B) over-weekend runs, and C) reduced hands-on time for solution providers. Scoring scale: 0-5; 0 = not important at all; 5 = very important; N/A = not applicable.

The answers also revealed a high importance given by solution providers to:

- paired-end sequencing
- flexibility of the platform
- pooling various libraries in one run
- IVDR-CE certified tests and instruments
- Long read sequencing (>600bp)

Considering the defined importance of lab-developed tests for the buyers group, it is worth noticing that 70% of the responders perform sequencing as a lab-developed test, and therefore this information should be considered in preparation for the Call for Tenders.

c) Lot 3 – Bioinformatics

Unfortunately, the low number of responses from users to the Lot 3 questions does not allow a direct comparison between existing needs and readiness of solution providers to address them. Nevertheless, the responses from the solution providers give us valuable insights to define Lot 3 specifications.

- Solutions for rare diseases were well represented by the participants, which is likely the field with the biggest needs in terms of bioinformatics analysis. The most common type of processed NGS data by these providers also matches well with the interest shown by the buyers group and by the users.

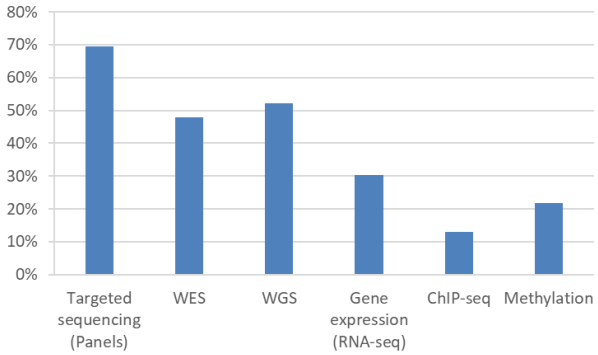


Figure 10. Type of NGS data covered by solution providers

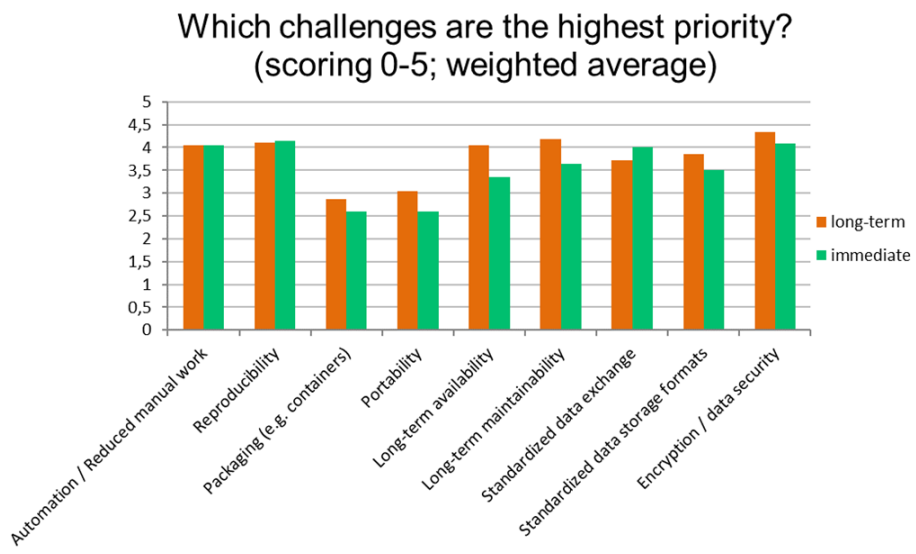


Figure 14. Bioinformatics challenges to overcome with highest priority. The weighted average score is displayed for users and solution providers (score 0-5). 0 = very low priority; 5 = very high priority.

- Key challenges related to storing and sharing of relevant data for diagnostic purposes:
    - data size and location of storage (GDPR), encryption, long-term storage, patient access and Global Alliance for Genomics and Health (GA4GH) standards.
- Key challenges ensuring secure handling of NGS data in diagnostics security updates:
    - compliance with GDPR requirements, training and awareness of personnel
- Key challenges to introduce standardized pipelines and software to detect actionable items for diagnostics purposes:
    - reproducibility, compatibility between tools or databases, complying with standard data formats and testing of pipelines with standardized samples

Furthermore, there is a strong need for solutions ensuring secure handling of NGS data in diagnostics security updates, where compliance with GDPR requirements, training and awareness of personnel appear to be the most important challenges to face.

d) Lot 4 – Integrated Reporting

- All users are interested in implementing tools for integrated reporting in routine praxis and most already use such tools
- There is a low satisfaction with existing products on the market, among both users and providers
- Currently available tools mainly integrate cancer-related genes and actionable items, whereas pharmacogenomic variants and evidence for variants are not yet well integrated
- The solution should be available on desktop devices, have graphical presentation of results and integration of the results in electronic health records
- Generating a special report for the patients is very important for users
- Users have a preference for local analysis, whereas providers prefer analysis via web service

Table 4 depicts a ranked list of the most important information to be included in the integrated reporting, according to answers given by users and solution providers.

Table 4. Ranking of the most important information to be included in integrated reporting solutions for decision-making. The weighted average score is displayed for users and solution providers. Scoring scale: 0-5; 0 = not important; 5 = highly important.

	USERS	SOLUTION PROVIDERS
Ranking	Challenge	Challenge
1	Results on cancer –related variants	Results on cancer – related variants
2	Information on the level of evidence for cancer-related variants	Information on actionable items
3	Information on the quality of the analysis	Information on the sample analyzed
4	Information on actionable items	Information on pharmacogenomics variants
5	Information on the sample analyzed	Information on the quality of the analysis
6	Information on the analytical method	Information on the analytical method
7	Information on running clinical trials	Information on the level of evidence for cancer-related variants
8	Information on possible compassionate use	Information on the level of evidence for pharmacogenomics variants
9	Information on drug-drug interaction, dosing, side effects and contra indications(e.g., e-medication)	Information on informed consent
10	Information on pharmacogenomics variants	Information on running clinical trials
11	Information on the level of evidence for pharmacogenomics variants	Information on possible compassionate use
12	Information on relevant clinical data (e.g., heart, liver, kidney function)	Information on relevant clinical data (e.g., heart, liver, kidney function)
13	Information on informed consent	Information on drug-drug interaction, dosing, side effects and contra indications(e.g., e-medication)

The valuable input and lessons learned from the broad community of stakeholders which participated in the OMC questionnaires, as summarized here, was responsibly considered for refining the specifications for the Call for Tenders. This includes the individual responses and open-field questions for all Lots. Together, the lessons learned from the OMC virtual meeting and the collected information from the questionnaires will be considered for the Lots' specifications and criteria and complete the clinical and patients's needs in the preparation of the documentation for the Instand-NGS4P Call for Tenders.